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MASTER

REVIEWS OF THE ENVIRONMENTAL EFFECTS OF POLLUTANTS:

X. Toxaphene

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REVIEWS OF THE ENVIRONMENTAL EFFECTS OF POLLUTANTS: X. TOXAPHENE

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1.0 SUMMARY

1.1 DISCUSSION OF FINDINGS

1.1.1 Chemical Properties and Analytical Techniques

Technical toxaphene is a synthetic organic chemical which is manufactured by chlorinating the diterpene, camphene, to a 67 to 69 percent chlorine content (Section 2.3). The technical mixture contains more than 175 polychlorinated diterpenes, none of which is present in more than a few percent (Section 2.4). Technical toxaphene is an amber, waxy solid which melts in a range from 65 to 90 C and has a vapor pressure of 0.17 to 0.4 mm Hg at 25 C. Its solubility in water is ca. 40 micrograms per liter, but it is soluble in most organic solvents (Section 2.2).

The chemistry of technical toxaphene is typical of the chlorinated paraffins. While it hydrolyzes slowly below pH less than 10, it rapidly reacts with strong alkali. It is reduced by active metals such as sodium and reacts with Lewis acids such as aluminum chloride. It shows little photochemical reactivity within the sunlight spectrum (Section 2.5).

Biochemical behavior has not been studied with technical toxaphene, but has been examined for two of the isolated toxaphene constituents: Toxicant A (a mixture of 2,5-_endo_, 2,6-_exo_, 8,9,9,10-octachlorobornane and 2,5-_endo_, 2,6-_exo_, 8,8,9,10-octachlorobornane) and Toxicant B (2,5-_endo_, 2,6-_exo_, 8,9,10-heptachlorobornane). Both toxaphene constituents reacted with biological iron protoporphyrin systems (in vitro) to yield reductively dechlorinated and dehydrochlorinated products (Section 2.5).

The recommended method for environmental toxaphene residue analysis is gas chromatography (GC) with electron capture or microcoulometric detection.

While GC with detection by mass spectrometry (specific ion monitoring) is more specific and sensitive than the conventional detectors, its cost is prohibitive for many laboratories. Less expensive (but also less sensitive) alternatives to GC are colorimetry (diphenylamine derivative) and chromatographic methods such as thin-layer chromatography (TLC) and reverse phase TLC (Sections 2.6.4.2 and 2.6.4.3).

Numerous interferences, including naturally occurring materials, other pesticides, and synthetic organic chemicals, can affect GC and other forms of analysis, with the possible exception of GC with mass spectral detection (Section 2.6.4.1.3). To prevent sample contamination (especially by organic plasticizers), glass equipment should be used for collecting and pretreating samples.

Air samples are collected by impingers, filters, and adsorbents. Polyurethane foam plugs are an excellent media for collecting atmospheric toxaphene (Section 2.6.2.1). With aqueous samples the best procedure is to extract toxaphene with a pure hydrocarbon solvent such as n-hexane (Section 2.6.2.2). Soils and sediments are extracted by a number of techniques; the two most popular are mechanical shaking and the Soxhlet extraction with solvent (hexane-acetone azeotrope is the preferred solvent) (Section 2.6.2.3). Biological samples (including foods) are liquified, if necessary, and extracted with hydrocarbon solvents. They often contain large concentrations of naturally occurring interferences, such as fats, oils, and pigments. These can be removed by hexane/acetonitrile partitioning, treating with strong acid, and/or column chromatography (Section 2.6.2.4). Several procedures are available for removing synthetic organic chemicals which interfere with toxaphene GC. Generally, these procedures include treatment with strong acid (e.g., fuming sulfuric), treatment with strong base, and column chromatography (Sections 2.5.2 and 2.6.3).

The individual chlorinated diterpenes which comprise the complex toxaphene mixture differ in their physical, chemical, and toxicological properties. Several laboratories have separated toxaphene into fractions based upon differences in the polarity, adsorption behavior, solubilities, and other properties of the individual components. Seven distinguishable constituents have been isolated in fairly pure form and their compositions and properties evaluated (Section 2.4.3). While one constituent is actually a mixture of two isomeric octachlorinated bornanes, all others have been identified as single compounds. The eight compounds which have been identified include seven polychlorinated bornanes and one polychlorinated dihydrocamphene. Environmental weathering apparently alters toxaphene composition due to differences in rates of chemical and biochemical degradation and removal by physical processes (e.g., evaporation, dissolution). However, GC chromatograms of the residues are still recognizable, although bioassay of these residues demonstrates that the toxicity has often been considerably changed. While the relationships between the change in composition and toxicity are important, they are largely unknown.

1.1.2 Environmental Occurrence

Toxaphene is a synthetic product, the only known source of which is commercial production. Therefore, any contamination results from release during production or commercial use of toxaphene. Approximately 50 million kg of toxaphene are annually produced in the United States (Section 8.2).

Soil and water monitoring studies (Sections 7.2.2 and 7.2.3) suggest that toxaphene is not as widespread an environmental contaminant as other chlorinated hydrocarbon insecticides, such as DDT and dieldrin. Toxaphene is rarely detected in soil, water, or sediment samples that have not received direct or nearby applications of the pesticide for crop protection (mostly cotton), rough fish control, or loss from a manufacturing plant. When detected, toxaphene water concentrations rarely exceed ppb levels, while sediment and soil samples usually are in the ppm range. This lack of widespread detection of toxaphene may be due, in part, to the difficulty encountered in trace analysis of such a complex mixture of compounds (Section 2.6) and the high volatility of toxaphene (Section 2.2). In contrast to the lack of water and soil contamination, toxaphene appears to be a widespread atmospheric contaminant. Sampling of western North Atlantic Ocean air indicates that the mean levels of toxaphene (0.7 to 0.8 ug/cu m) are equal to or twice those of PCB's and ten times higher than those of other pesticides such as DDT (Section 7.2.1). This high concentration probably results from both the large scale use of toxaphene and its volatility.

1.1.3 Environmental Cycling and Fate

A quantitative understanding of the cycling of toxaphene is not available, but some field and monitoring studies provide some insight into the processes that take place. Much of the toxaphene which is released into the environment will evaporate at a relatively fast rate because of its high volatility. Toxaphene in the atmosphere (in the vapor state or adsorbed to particulate matter) can travel long distances. Toxaphene residues in air have been detected near the island of Bermuda, 1200 km from the southeastern cotton belt (Section 7.2.1). Part of the toxaphene that is sprayed on crops will land on the foliage or soil. The more volatile toxaphene components will evaporate from the foliage, while the less volatile components are washed onto the soil (Section 8.3). Toxaphene residues in soil can evaporate, be carried by erosion to rivers and lakes when adsorbed to small particles of soil (less than 1 percent of applied toxaphene is found in runoff - Section 7.3.3), or leach into ground water. Most studies have found very little soil leaching, although one investigation demonstrated that toxaphene could leach through three feet of soil (Section 7.3.3). Toxaphene residues in water have a tendency to adsorb to suspended particles or sediment, which explains the low (ppb) concentrations found in water (Section 7.3.2).

Degradation of toxaphene in the environment is poorly understood. Toxaphene is not susceptible to hydrolysis or photolysis (Section 7.3.2) but does appear to biodegrade in soil under anaerobic conditions (Section 7.3.3). Several researchers have noted that longer retention time (less volatile) peaks in the gas chromatogram of soil and water toxaphene residues decrease, while shorter retention time (more volatile) peaks increase. Residues from water and sediment do not appear to be products of dehydrohalogenation (Section 7.3.2). The gas chromatograms of atmospheric toxaphene residues are only slightly changed.

The rate of environmental degradation can vary considerably. One study determined that the concentration of toxaphene in lake sediment decreased by a factor of 2 every 20 days. However, concentrations of toxaphene in lakes treated 3 to 9 years before monitoring contained 1 to 4 micrograms per lite in water and 0.2 to 1 ppm in sediment (Section 7.3.2). The reported half-residence time for toxaphene in soil varies from 100 days to 11 years (Section 7.3.3). The latter is considered a maximum value.

1.1.4 Biological Aspects in Microorganisms

Toxaphene is not toxic to soil bacteria and fungi or to the microbiological processes important to soil fertility at concentrations even higher than those used for controlling soil insects (Section 3.2.3.3). Inhibition of growth and photosynthesis by toxaphene at concentrations lower than 1 ppm has been observed in many species of fresh water and marine unicellular alga (Section 3.4.2). No single toxaphene fraction is exclusively responsible for toxicity. Toxaphene is stimulatory to growth, carbon fixation, or cell population of algae, soil bacteria, and fungi at very low concentrations.

The uptake and metabolism of toxaphene by microorganisms has not been adequately studied. The available data show that microorganisms do not bioaccumulate significant quantities of the pesticide from the medium (Sections 3.2.1, 3.3.1, 3.4.1). No degradation of toxaphene by microorganisms has been noted.

1.1.5 Biological Aspects in Plants

The uptake and metabolism of toxaphene by plants has not received much investigation. Aquatic plants are able to accumulate appreciable quantities of toxaphene from the medium (Section 4.3.1.1). Salt marsh (cod grass) collected from a contaminated estuary contained as much as 36.3 ppm toxaphene in leaves. Significant toxaphene residues have also been detected in many crop plants (Section 4.3.2.1) when toxaphene is directly applied (rarely occurs commercially). The residues were higher in most crops immediately after harvest, but decreased upon incubation. Whether crop residues resulting from direct application are due to surface deposition or to translocation of toxaphene in plants is unclear, although the former is thought to be the most important. Toxaphene residues are reduced by commercial packing house washing and during food processing (4.3.2.1). Exposure to toxaphene via food is not appreciable as demonstrated by the FDA market basket survey. Though toxaphene is not recommended for use in tobacco, residues have been detected in tobacco and tobacco products.

Toxaphene is not toxic to most crop plants at concentrations recommended for control of insects (15 to 20 kg/ha, annually) (Section 4.3.2.3). Some adverse affect has been noted in the case of cauliflower, cabbage, and tomatoes. Toxaphene causes off-flavor in tobacco when applied to tobacco foliage. No information concerning the effect of toxaphene on non-crop plants is available.

1.1.6 Biological Aspects in Wild and Domestic Animals

Both aquatic and terrestrial animals are able to absorb and store toxaphene in significant quantities. Quantitative data, however, are available only on aquatic species. Fish and aquatic invertebrates are able to concentrate toxaphene from water by factors of several thousand (Sections 5.2.1.1 and 5.3.1). Although model ecosystem studies indicate that biomagnification may occur (Section 8.4.3), one field study has shown that predator fish accumulate less toxaphene than prey fish (Section 5.2.1.5). While this finding might suggest different rates of toxaphene biotransformation and/or elimination in these two groups of fish, experimental data on toxaphene metabolism by aquatic species is inconclusive. Less is known about the uptake and elimination of toxaphene in terrestrial animals. Based on residue analyses, birds seem to be able to absorb toxic quantities of toxaphene from contaminated food (Section 5.4.1.5). Similar studies in domestic animals have shown that toxaphene may be absorbed from either oral or dermal exposures with detectable residues being found in the meat or milk (Section 5.5.1.5). In two species of cockroach, toxaphene has been shown to penetrate into the hemolymph. While no evidence was found for toxaphene metabolism in the cockroach, a toxaphene dehydrochlorinase has been reported in the cotton leafworm (Section 5.6.1).

The acute toxicity of toxaphene varies within each of the animal groups on which data are available. However, in both aquatic and terrestrial animals, the variability is much more prominent in the invertebrates than in the vertebrates. In fish, acute LC50 values vary by a factor of about ten, ranging from 3 ppb to 32 ppb (Section 5.2.2.2.1). In aquatic invertebrates, LC50 values vary by a factor of two and one-half million, ranging from 7.2 ppt to 18 ppm (Section 5.3.2.2.2). A similar, though less pronounced pattern, is seen in terrestrial animals. Acute oral LD50 values for birds range from 10 to 300 mg/kg (Section 5.4.2.2.1). These estimates are quite similar to the oral LD50 values seen in experimental mammals - i.e., 25 to 270 mg/kg (Section 6.3.2.1.1.1). In insects, acute topical LD50 values range from 5.43 mg/kg to 1789 mg/kg (Section 5.6.2.2). While this marked variability in invertebrate forms is probably related to the increased phylogenetic diversity of these animals, the specific biological mechanisms involved - e.g., different rates of absorption or detoxication - are not known.

Based on the available chronic toxicity data, aquatic organisms may be highly susceptible to toxaphene contamination. In water, concentrations of toxaphene as low as 0.055 ppb have been shown to cause increased backbone fragility in fathead minnows over exposure periods of 150 days. Toxaphene concentrations of 0.39 ppb over 60 day exposure periods also cause decreased growth in brook trout. Pathological changes in fish chronically exposed to toxaphene include degenerative changes in liver, kidney, gill, digestive tract, and nerve tissue (Section 5.2.2.2.2). While less detailed information is available on chronic toxic effects in aquatic invertebrates, toxaphene at 1 ppb has been shown to depress growth in oysters. However, field studies on aquatic invertebrate populations suggest that toxaphene concentrations of 0.070 ppb to 0.300 ppb have no effect on these organisms. Less extensive data are available on terrestrial organisms. In pelicans, toxaphene given in the food at 50 ppm for three months was lethal. However, in ring-necked pheasants, toxaphene at 300 ppm in the diet given over the same period had no marked toxic effect (Section 5.4.2.2.2). In the only subacute study found on domestic animals, young steers given average doses of toxaphene at 7.9 mg/kg for four months show evidence of transient nervous system effects (Section 5.5.2.2.2).

Chronic exposure to toxaphene may also affect the reproductive capacity of many different types of animals. Decreased egg viability has been noted in spawning female brook trout exposed to 0.075 ppb toxaphene. Developmental abnormalities have been seen in striped mullet embryos exposed to 500 ppb toxaphene, and high mortality was found in clam larvae exposed to 250 ppb toxaphene (Sections 5.2.2.2.3 and 5.3.2.2.4). However, some field studies on toxaphene treated lakes indicate that toxaphene does not inhibit fish productivity at concentrations of up to 1 ppb. Dietary concentrations of 300 ppm toxaphene have resulted in decreased reproductive success in ring-necked pheasants. However, toxaphene has not been shown to affect egg shell thickness in quail given single oral doses of 10 mg/kg, nor does it affect hatchability of hens' eggs injected with 1.5 mg toxaphene/egg (Section 5.4.2.2.3). In insects, egg production failure was seen in a population of lady beetles after a single application of toxaphene at 10 micrograms per insect, but no effect on productivity was observed in milkweed bugs exposed to sublethal levels of toxaphene for 17 generations (Section 5.6.2.2.4).

The long term effects of toxaphene may be substantially influenced by the development of resistance. True genetic resistance has been most extensively documented in the terrestrial insects. Various species of lepidoptera, coleoptera, diptera, hymenoptera, and hemiptera have been shown to possess resistance to acute toxaphene poisoning. For the most part, resistant insect strains are able to tolerate doses of toxaphene ten times above those causing high mortality in corresponding susceptible strains. Although the ability to develop resistance to toxaphene may be inherent in most insect species, the extent to which resistance does develop may be quite limited. For instance, in a study on boll weevils, a 10 to 12-fold resistance to a 2:1 toxaphene-DDT mixture developed during a 22 generation exposure period. However, no increase in resistance developed over an additional 20 generation exposure period (Section 5.6.2.2.3). Resistance has also been demonstrated in fish. In these animals, resistance factors of up to 200 have been noted. However, in most instances, resistant strains are about 40 to 70 times less sensitive to toxaphene than susceptible strains, and no clear distinction is made between true genetic resistance and toxaphene tolerance (Section 5.2.2.2.4). Although there is no reason to presume that toxaphene resistance will not develop in aquatic invertebrates, only one report of such resistance is available. In this study, a strain of fresh water shrimp was found to be resistant to toxaphene by a factor of ten. As in many of the fish studies, no clear distinction was made between resistance and tolerance (Section 5.3.2.3).

1.1.7 Biological Aspects in Humans and Test Animals

Possible sources of human exposure to toxaphene include contaminated water, food, or air. Monitoring studies suggest that water and food rarely have detectable levels of toxaphene, except when toxaphene is applied or spilled nearby the site of contamination. During localized spills, toxaphene can bioconcentrate in fish that may be consumed by humans. Market basket surveys by FDA, which include fish, indicate that this type of contamination rarely occurs. In contrast, human exposure due to contaminated air appears to occur routinely, based upon monitoring data.

Toxaphene can be absorbed across the alimentary tract, skin, and respiratory tract. Like most lipophilic chlorinated pesticides, toxaphene is stored primarily in fatty tissue. Although the metabolism of toxaphene in mammals has not been described in detail, dechlorination mediated by the microsomal mixed-function oxidase system seems to be a major route of detoxication. In rats, the biological half-life of orally administered toxaphene is between 1 and 3 days, with elimination taking place via the urine and feces (Section 6.2).

The best estimates of the minimum acute oral lethal dose for man vary between 30 and 103 mg/kg. These estimates are close to both the acute oral LD50 values for various laboratory mammals (25 to 220 mg/kg) and, as mentioned in Section 1.1.6, to the acute oral LD50 values for birds (10 to 300 mg/kg). The acute dermal toxicity of toxaphene is about an order of magnitude less than that of oral toxicity. Regardless of the route of administration, the signs of acute toxaphene intoxication are similar in all mammals. Central nervous stimulation, evidenced by the development of clonic-tonic convulsions, is consistently noted. Additional signs include apprehensive behavior, salivation, vomiting, and hyperreflexia. Exposure to toxaphene-lindane mixtures has been associated with the development of acute aplastic anemia in man (Section 6.3.2.1).

The subacute toxicity of toxaphene has not been extensively investigated. In humans, prolonged occupational exposure to toxaphene vapors may cause respiratory insufficiency and reversible lung damage. In one study with rats, dietary levels of toxaphene at 50 ppm for 2 to 9 months have been associated with hydropic accumulation of the liver. Other studies, however, have failed to confirm this effect (Section 6.3.2.2).

By far the most important studies on toxaphene toxicity are concerned with toxaphene carcinogenicity, teratogenicity, and other reproductive effects. Results of a study by the National Cancer Institute indicate that toxaphene fed to mice at levels of about 100 ppm over a one and one-half year period causes a high rate of hepatocellular carcinoma. In rats, toxaphene was associated with an increase in the incidence of thyroid tumors (Section 6.3.2.4).

The teratogenic and reproductive effects of toxaphene are less severe. In rats, toxaphene doses which caused high maternal mortality were associated with an increased incidence of encephaloceles. However, at lower doses, toxaphene does affect implantation frequency, fetal mortality, and fetal weight gain in rats and mice, and results in decreased numbers of ossification centers in rats (Section 6.3.2.6). Based on the dominant lethal assay in mice, toxaphene does not appear to be mutagenic. However, mutagenicity has been demonstrated in several bacterial assays (Section 6.3.2.5).

1.1.8 Food Chain Interactions

Human ingestion of toxaphene in food is apparently low as demonstrated by the annual market basket survey conducted by the Food and Drug Administration (Sections 8.4.1 and 4.3.2.1). This is probably due to the fact that (1) toxaphene is mostly used on cotton crops, and (2) washing and food processing remove considerable amounts of toxaphene (Section 4.3.2.1). Any residues that have been detected on crops are thought to be due to surface deposition rather than uptake from soil, although this has not been well demonstrated experimentally.

Little information on adsorption and distribution of toxaphene in birds and terrestrial wildlife is available, but residues have been detailed in some species (especially fish-eating birds) (Section 8.4.2). Controlled exposure studies have demonstrated that cows fed toxaphene in their diet would have residues in their milk.

In aquatic ecosystems, toxaphene bioaccumulates in plants, bacteria, fungi, green algae, oysters, plankton, daphnia, and fish (Section 8.4.3). In fish, toxaphene is bioconcentrated by a factor of several thousand. Field studies show that prey fish stocked in a toxaphene-treated lake concentrate more toxaphene than predator fish, which suggests that biomagnification may not be an important process although other explanations are possible (Section 8.4.3).

1.2 CONCLUSIONS

1. Toxaphene is a mixture of more than 175 polychlorinated diterpenes which are water insoluble, stable to hydrolysis at pH less than 10 but react rapidly with strong alkali, reduce with active metals, and are stable under sunlight irradiation.

2. Using gas chromatography with appropriate clean-up procedures, toxaphene concentrations of 1 ppb can be detected.

3. The major source of environmental release of toxaphene is from its application to cotton as an insecticide.

4. Toxaphene residence time in soil and water is considerably less than other chlorinated hydrocarbon pesticides, such as DDT, but detectable levels (0.7 to 0.8 ng/cu m) are found in the atmosphere at sites remote from points of application, suggesting that the overall environmental persistence is quite high. Little is known about the environmental fate of toxaphene, one of the highest volume pesticides presently in use.

5. Toxaphene is not toxic to plants, soil bacteria and fungi, or to the microbiological processes important to soil fertility. Growth and photosynthesis in unicellular-fresh water and marine algae are inhibited by toxaphene. Uptake and metabolism of toxaphene by plants and microorganisms have not been adequately investigated.

6. Degradation of toxaphene by pure cultures of microorganisms has not been observed. Microorganisms are unable to concentrate toxaphene to a significant extent from the medium.

7. Exposure of humans to toxaphene via food does not appear to be appreciable. Fish can bioconcentrate toxaphene, but market basket surveys, which include fish, indicate that contamination is not widespread.

8. Aquatic organisms are highly sensitive to technical toxaphene. At concentrations of 1 ppb and below, laboratory studies indicate that toxaphene may lead to diminished reproductive capacity, decreased growth, and skeletal defects. None of these results have been confirmed in field studies.

9. Dietary levels of toxaphene above 50 ppm can be lethal or can cause decreased reproductive capacity in some species of birds. Such exposures can occur during the use of toxaphene as an insecticide or piscicide.

10. At dietary levels of 100 ppm, toxaphene is apparently carcinogenic to mice and possibly rats. Whether the concentrations routinely found in air will cause cancer, is unknown.

11. Toxaphene has been shown to be mutagenic in microbial assay systems.

12. Toxaphene bioconcentrates in many aquatic organisms. In fish, concentrations several thousand times the concentration in water are found in laboratory and field studies.

2.0 CHEMICAL AND PHYSICAL PROPERTIES AND ANALYSIS

2.1 SUMMARY

Technical toxaphene is a mixture of more than 175 polychlorinated diterpenes, all of which are present in concentrations of a few percent or less. It is produced by chlorinating camphene to a 67 or 69 percent chlorine content. Toxaphene is similar to a number of other chlorinated diterpenes, the most important of which is Strobane, a suspected carcinogen. Strobane is prepared by chlorinating α-pinene to 66 percent chlorine content. Since toxaphene and Strobane contain many chromatographic peaks of identical retention time and yield similar mixtures of hydrocarbons when they are reduced, they probably contain many of the same polychlorinated species.

Overall, the physical and chemical properties of toxaphene and its individual constituents are poorly defined. Technical toxaphene is a waxy solid with a broad melting range (65 to 90 C), moderate vapor pressure (0.17 to 0.4 mm Hg at 25 C), and low solubility in water (ca. 40 micrograms/liter). Its chemistry is typical of chlorinated paraffins: it hydrolyzes very slowly in aqueous acid or neutral water but rapidly reacts with strong aqueous alkali, probably by dehydrohalogenating; it is reactive with Lewis acids, such as aluminum chloride; it is reduced by active metals, such as sodium; and it is not photochemically reactive at sunlight wavelengths.

Properties of the individual constituents apparently differ, based upon observations of technical toxaphene. Numerous studies on toxaphene separation have demonstrated that the constituents differ in polarity, adsorption behavior, and solubilities in a number of solvents. Field studies on applied toxaphene and its residues all indicate that the constituents differ in vapor pressures.

aqueous solubilities, chemical reactivities, and other properties. Only eight constituents have been isolated and identified, of which seven are chlorinated bornanes and one is a chlorinated dihydrocamphene. The melting points, spectral properties, and toxicities of the individual constituents are different. The only work on chemical behavior of the isolated constituents has shown that Casida's Toxicants A (a mixture of 2,5-endo, 2,6-exo,8,8,9,10-octachlorobornane and 2,5-endo, 2,6-exo,8,9,9,10-octachlorobornane) and B (2,5-endo, 2,6-exo,8,9,10-heptachlorobornane) react with biological iron protoporphyrin systems in vitro to yield reductively dechlorinated and dehydrochlorinated products. However, relative rates and yields for these reactions and products are unknown.

The differential reactivities and physical properties of toxaphene constituents make the study of technical toxaphene and its residues more difficult than other chlorinated hydrocarbon insecticides, such as DDT or aldrin. This is manifested both in the analysis of toxaphene residues in the environment and in the interpretation of hazards of the residues.

The recommended method for environmental toxaphene residue analysis is by gas chromatography (GC) with electron capture or microcoulometric detection. Detection by mass spectrometry (specific ion monitoring) is more specific and sensitive for toxaphene residue detection, but its cost is prohibitive for many laboratories. Less sensitive alternatives for analysis are available, such as colorimetry (diphenylamine method) and thin-layer chromatography or related methods (e.g., reverse-phase thin-layer chromatography). Because toxaphene yields a large number of GC peaks over a broad range of retention times, numerous interferences can affect its analysis. Analytical methods must either remove or correct for possible interferences.

While environmental weathering alters the composition of toxaphene and the GC pattern of its residues, the pattern can generally be recognized. The composition change apparently results when constituents are degraded or removed (evaporation, dissolution, etc.) at different rates. Bioassay has shown that the weathered residues are no longer toxic. However, the relationships between the ambient environmental toxaphene residues, environmental transformations and losses of toxaphene constituents, and the acute and chronic toxicities of the weathered residues have not adequately been examined.

2.2 PHYSICAL CHARACTERISTICS OF TECHNICAL TOXAPHENE

Toxaphene is defined as chlorinated camphene with a combined chlorine content of 67 to 69 percent (Guyer et al., 1971; Hartwell et al., 1974). This complex mixture consists of more than 175 polychlorinated diterpenes (Section 2.4). The most numerous and important of these constituents are the polychlorinated bornanes and dihydrocamphenes. The structures and numbering sequences for the bornane and dihydrocamphene systems are illustrated in Table 2.1.

Table 2.1 enumerates the typical properties and Table 2.2 lists the product specifications of commercially available toxaphene. Technical toxaphene is an amber, waxy solid with a vapor pressure ranging from 0.17 to 0.4 mm Hg at 25 C. The broad melting range (65 to 90 C) characterizes it as a mixture rather than a pure compound. While it is only slightly soluble in water, it is soluble in and can be recrystallized from a number of organic solvents (Black, 1974; Khalifa et al., 1974; Anagnostopoulos et al., 1974).

The literature has limited information on the physical properties of the individual constituents of toxaphene. Section 2.4.3 describes recent work published on the isolation of several (eight) constituents. The only physical

Table 2.1. Physical properties of technical toxaphene[a]

Emperical formula: $C_{10}H_{10}Cl_8$

Average molecular weight: 414

Percent chlorine content: 67 to 69%

Physical form: amber, waxy solid

Melting point: 65 to 90 C

Vapor pressure (mm Hg): 0.17 to 0.4 at 25 C
3 to 4 at 90 C

Specific gravity at 100 C/15.6 C: 1.63 (average)

Specific gravity change per degree C: 0.0012

Pounds per gallon at 75 C: 13.8

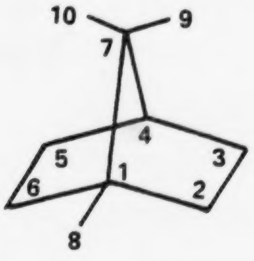
Viscosity (Centipoises): 89 at 110 C
57 at 120 C
39.1 at 130 C

Specific heat (cal/g): 0.258 at 41 C
0.260 at 95 C

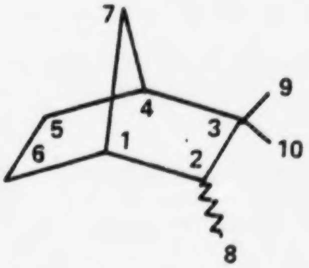
Solubility: 37 microgram/liter[b] in water at ambient temperature. Highly soluble in most organic solvents; greater solubility in aromatic solvents

Octanol/water partition coefficient:[c] 825

Chemical Structures



Bornane



Dihydrocamphene
(Camphane)

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[a] Sources: Adapted from Hartwell et al., 1974; Guyer et al., 1971; Hughes, 1970.

[b] From Lee et al. (1968). Many reviews list the water solubility at 0.4 mg/liter based on work by Cohen et al. (1960).

[c] From Sanborn et al. (1976).

Table 2.2. Toxaphene product specifications[a]

Total organic chlorine, % by weight:	67.0-69.0
Acidity, % by weight as HCl:	0.5 maximum
Drop softening point, C:	70 minimum
Infrared absorptivity at 7.2 micron:	0.0177 maximum
Specific gravity at 100 C/15.6 C :	1.600 minimum

[a] Source: Hartwell et al., 1974

property measured for the components was their melting points, which are substantially higher than the melting range for technical toxaphene. Differences in other physical properties of toxaphene constituents are apparent but have not been quantitatively measured. The evidence for these differences comes from observations on the properties and behavior of technical toxaphene. Numerous studies on toxaphene separation have observed differential solubilities, polarities, and adsorptive characteristics of the constituents (Black, 1974; Seiber et al., 1975; Holmstead et al., 1974; Anagnostopoulos et al., 1974). Seiber et al. (1975) has cited vapor pressure differences as the cause of compositional changes in residues for the environmental weathering of field applied toxaphene. While differences in other physical properties are expected to influence the changes in the composition of toxaphene residues exposed to the environment, this logical conclusion has neither been quantified nor tested in the laboratory or field.

2.3 TOXAPHENE PRODUCTION

2.3.1 Manufacturing Processes

Toxaphene is manufactured by chlorinating camphene to 67 to 69 percent chlorine content. Figure 2.1 describes the process flow. Camphene is derived from turpentine, which is either a product of sulfate turpentine from the Kraft paper process or wood turpentine from the extraction of pine stumps (Guyer et al., 1971; Hartwell et al., 1974; Buntin, 1951, 1974; Sittig, 1967, Lawless et al., 1972; von Rumker et al., 1974). The turpentine, which contains primarily α-pinene, is catalytically isomerized to camphene and subsequently purified by fractionation (Buntin, 1974). While the chlorination grade camphene may contain some tricyclene, less than 5 percent of other terpenes is present (Guyer et al., 1971). The chemistry of the production process is detailed in Section 2.3.2.

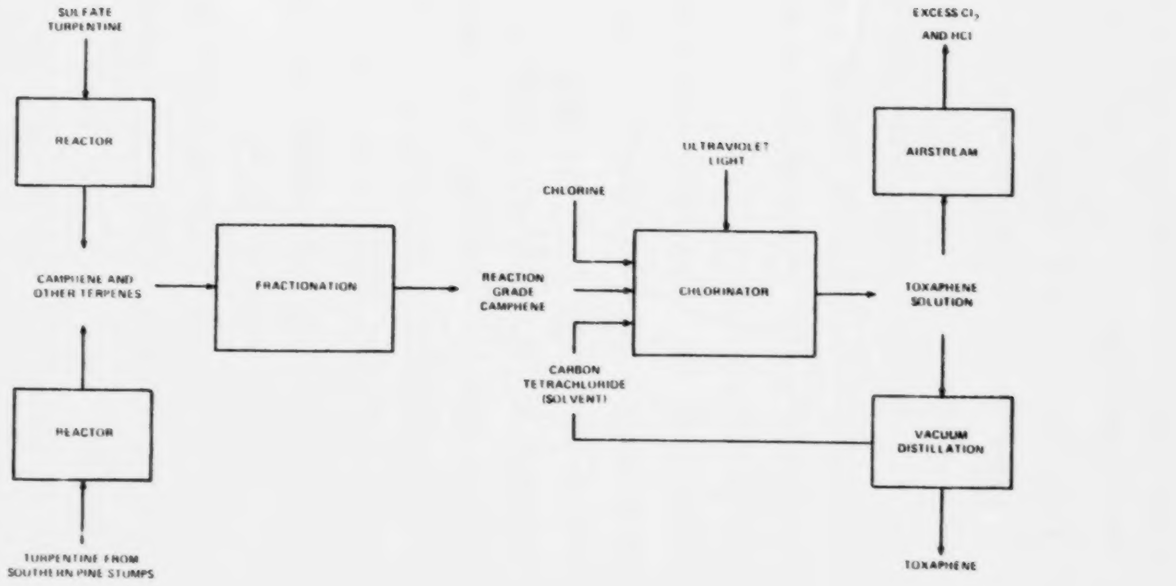

SULFATE TURPENTINE
REACTOR
CAMPHENE AND OTHER TERPENES
REACTOR
TURPENTINE FROM SOUTHERN PINE STUMPS
FRACTIONATION
REACTION GRADE CAMPHENE
CHLORINE
CARBON TETRACHLORIDE (SOLVENT)
ULTRAVIOLET LIGHT
CHLORINATOR
EXCESS Cl_2 AND HCl
AIRSTREAM
TOXAPHENE SOLUTION
VACUUM DISTILLATION
TOXAPHENE

Figure 2.1. Schematic flow chart for toxaphene manufacture. Sources: Lawless et al., 1972; von Rumker et al., 1974.

The process feed consists of a carbon tetrachloride solution of camphene containing about five parts solvent to one part camphene (Sittig, 1967; Buntin, 1974). The reactor is a lead-, glass-, or nickel-lined vessel equipped with a heat-exchange jacket, a reflux condenser, and a well for an ultraviolet lamp. The reaction is carried out at atmospheric pressure and the initial reaction temperature is maintained at 85 to 90 C, which requires some cooling. During the latter chlorination stages the temperature ranges from 50 to 75 C. The reaction requires from 15 to 30 hours (Sittig, 1967; Buntin, 1974). The product work-up consists of blowing out HCl and excess chlorine and then distilling carbon tetrachloride in vacuo.

Although toxaphene is a complex mixture, good quality control has apparently maintained a consistent product. Each commercial toxaphene batch is bioassayed (usually with houseflies, but sometimes with other test organisms) and physically and chemically analyzed to insure conformation with the product specifications (Hartwell et al., 1974). The infrared absorbance (at 7.2 microns) is used to distinguish toxaphene from other polychlorinated terpenes, such as Strobane (Hartwell et al., 1974; Guyer et al., 1971). Hartwell et al. (1974) report that electron capture gas chromatography (Section 2.6.4.1) is used to check the composition of each batch. Guyer et al. (1971) report that nine samples of toxaphene retained from production at Hercules, Inc., during the period from 1949 to 1970 were analyzed by the above methods and compared. Since results were consistent, they concluded that technical toxaphene has remained consistent in its composition and properties over the many years that it has been produced by Hercules, Inc. Whether technical products from other manufacturers are as consistent is unknown.

Other polychlorinated terpenes are manufactured by essentially identical procedures except that the composition of the starting material and the extent of chlorination are usually different (Melnikov, 1971; Sittig, 1967). Strobane, which is the major polychlorinated terpene that is analogous to toxaphene, is prepared by chlorinating turpentine (which contains mainly α-pinene) to approximately a 66 percent chlorine content.

Although the pure technical toxaphene appears to have remained consistent in its composition and properties over the years, there have been some problems of inconsistency in toxaphene formulation for animal spray and dipping vat preparations (Sparr et al., 1952; Radeleff and Bushland, 1960). These problems occurred when unequal concertrations of the insecticide developed during animal treatment with wettable powders and emulsifiable concentrates. This resulted in inadequate pest control in some of the treated animals and toxicosis in others. These formulation problems were solved in the early 1950's and, except for an occasional misuse on livestock, do not represent a hazard to the animal industry.

2.3.2 Chemistry of Toxaphene Production

Chlorination of camphene proceeds in two stages: (1) initial ionic addition of chlorine to unsaturated bonds followed by (2) free radical chlorination. The course of the ionic chlorination has been partially detailed by chlorinating camphene in the dark (Black, 1974; Richey et al., 1964, 1965; Jennings and Hershbach, 1965). Figure 2.2 describes the products of the ionic reactions. The major, initial products include exo-2,10-dichlorobornane, 3 (60 to 65 percent); a mixture of cis- and trans-8-chlorocamphene, 2 (19 percent); and exo-6-chlorocamphene, 5 (18 percent), which is a degradation product of 10-chlorotricyclene, 4 (Richey et al., 1964, 1965; Jennings and Hershbach, 1965). Black (1974) reports that subsequent ionic chlorination of 2, 4, and 5 yields cis- and trans-isomers

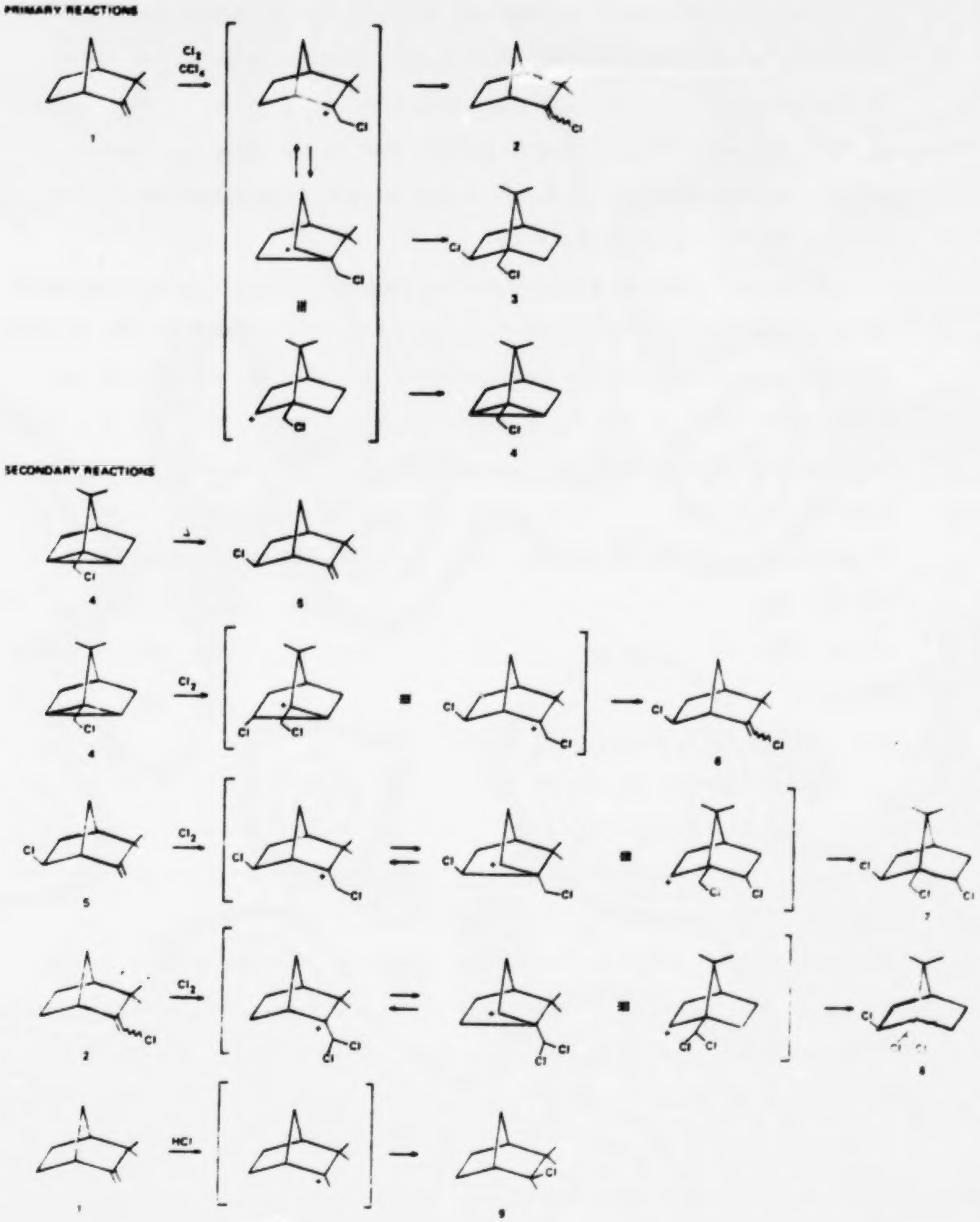

PRIMARY REACTIONS
Cl2
CCl4
1
2
3
4
SECONDARY REACTIONS
Δ
4
5
Cl2
6
5
7
2
8
HCl
1
9
Cl

Figure 2.2. Ionic pathways in the chlorination of camphene. Sources: Black, 1974; Seiber et al., 1975 ; Landrum et al., 1976; Jennings and Hershbach, 1965; Richey et al., 1964, 1965.

of exo-6,8-dichlorocamphene, 6; exo-2, endo-6,10-trichlorobornane, 7; and exo-2,10,10-trichlorobornane, 8.

Products of ionic chlorination are listed in Table 2.3. Some inconsistencies in the compositions reported by Black (1974) and previous work by Richey et al. (1964, 1965) and Jennings and Hersbach (1965) are apparent but have not been explained.

Recent work (Seiber et al., 1975; Landrum et al., 1976) suggests that hydrogen chloride, which is produced in the initial phase of the industrial chlorination, adds to camphene to yield exo-3-chlorocamphene, 9. The assignment of this reaction and product was based upon the isolation and identification of a dihydrocamphene derivative from technical toxaphene (Section 2.4.3).

The mono-, di-, etc. polychlorinated camphenes and bornanes formed by ionic chlorination are subsequently chlorinated by free radical pathways (Hughes, 1970; Black, 1974; Nelson and Matsumara, 1975 a). The free radical chlorination apparently follows the pathway:

$$Cl_2 \xrightarrow{h\nu} 2Cl$$

$$RH + Cl\cdot \longrightarrow R\cdot + HCl$$

$$R\cdot + Cl_2 \longrightarrow RCl + Cl\cdot$$

There has been no published information to describe the preferred site of chlorination or skeletal rearrangement of the free-radicals analogous to the Meerwein shifts (rearrangement of the norbonyl structure) of the corresponding cations.

Nelson and Matsumara (1975 a) examined the products from the free-radical chlorination of the major ionic addition product, exo-2,6-dichlorobornane.

Table 2.3. Composition of the products from ionic chlorination of camphene[a]

Compound[b]	Percent
Camphene(1)-tricyclene	6-8
6-Chlorobornane(5)	16-18
cis/trans 8-chlorobornene(2)	10-11
cis/trans 6,8-dichlorobornene (6)	11-12
Unknown dichlorocamphenes	2-3
2,10-Dichlorobornane(3)	26-27
2,10,10-Trichlorobornane(8)	10-12
2,6,10-Trichlorobornane(7)	1-2
2,9,10-Trichlorobornane	2-3
Total	84-96

[a] Source: Modified from Black, 1974.
[b] See Figure 2.2 for structure of the compounds.

The product was reportedly 1.7 times more toxic to certain insects than toxaphene. The gas chromatographs of the chlorination product of exo-2,6-dichlorobornane and toxaphene are similar, although the relative heights of some peaks differ.

2.4 COMPOSITION OF TOXAPHENE

2.4.1 Number and Molecular Formula of Constituents in Toxaphene

Literature previous to 1974 suggested that toxaphene contained 30 to 40 principal constituents (Hartwell et al., 1974; Guyer et al., 1971). However, more recent work discloses that it contains more than 177 individual polychlorinated terpenes containing from five to eleven chlorines. None of the constituents appear in concentrations greater than a few percent.

Holmstead et al. (1974) have published the most rigorous study on toxaphene composition. They used a combination of column chromatography (CC) and gas chromatography (GC)-mass spectroscopy (MS) to measure the molecular formula for individual constituents. The GC pattern of technical toxaphene is too complex to allow measurement of the MS of individual constituents. However, when technical toxaphene is fractionated by CC, the GC pattern of each fraction is simplified so that the MS of each peak can be measured. Apparently, GC separation of the components is dependent upon different properties than the CC separation (Section 2.4.3).

Holmstead et al. (1974) eluted recrystallized toxaphene on a silica gel column with hexane and initially collected over 700 fractions. They combined these initial fractions to yield a total of 18 fractions which were characterized by relatively distinct GC properties (Dexil-300 on Varaport-30 column). The molecular formula for each component was evaluated from the GC-MS by using the [M-Cl] positive ion peak for each component. Table 2.4 lists the apparent

Table 2.4. Components in toxaphene separated by silica gel adsorption chromatography and then gas chromatography with qualitative and quantitative analysis by chemical ionization mass spectrometry[a]

Number	Chromatographic properties: Silica gel fraction	Chromatographic properties: Gc R_t, min	Amount in toxaphene (%)
$C_{10}H_{12}Cl_6$, $[M - Cl]^+$ = m/e 307			
1	2	9.1	0.02
2	16	15.6	0.92
3	16	15.8	0.52
$C_{10}H_{11}Cl_7$, $[M - Cl]^+$ = m/e 341			
4	17	9.9	0.47
5	1	10.7	0.60
6	1-2	11.8	0.45
7	12	12.1	0.06
8	17	12.2	0.48
9	6	12.4	0.04
10	3-4	12.9	0.45
11	3	13.3	0.05
12	5	13.4	0.44
13	7-9	14.0	0.45
14	5-7	14.2	0.74
15	6-8	14.9	1.25
16	6	15.0	0.46
17	12	15.2	0.16
18	10	15.3	0.97
19	17	15.3	0.30
20	8	15.4	0.10
21	6	15.5	0.60
22	17	15.8	0.65
23	8-9	15.9	0.17
24	14	16.1	0.47
25	10-12	16.3	0.61
26	8-9	16.3	0.80
27	14-16	16.4	1.58
28[b]	10-14	16.7	2.48
29	15	16.9	1.05
30	12-13	17.3	0.90
31	13	17.7	0.30
32	17	21.1	0.65
$C_{10}H_{10}Cl_8$, $[M - Cl]^+$ = m/e 375			
33	4	11.1	0.07
34	2	11.3	0.03
35	4	11.3	0.07
36	1-2	12.0	0.24
37	2	12.4	0.13
38	1-2	12.7	0.32
39	1-2	13.3	0.76
40	4	13.3	0.21

---

[a] Source: Holmstead et al., 1974.

[b] Toxicant B - see Section 2.3.3.

[c] Toxicant A - see Section 2.3.3.

Table 2.4 (continued)

Number	Chromatographic properties Silica gel fraction	 Gc R_t, min	Amount in toxaphene (%)
41	1	13.8	0.09
42	4	13.8	0.06
43	3-5	14.6	1.06
44	1	14.7	0.56
45	17-18	14.8	0.96
46	4-5	15.0	0.17
47	11	15.3	0.21
48	7	15.4	0.27
49	17-18	15.5	0.74
50	6-7	15.7	0.39
51	18	16.1	0.40
52	1-2	16.2	0.98
53	6-7	16.3	0.21
54	2-4	16.5	1.32
55	5-7	16.7	0.70
56	9	16.7	0.09
57	17-18	16.9	0.84
58	2-4	17.1	1.46
59	8-11	17.1	0.56
60	14	17.1	0.26
61	8	17.4	0.08
62	14-16	17.4	2.40
63	2-4	17.5	1.42
64	11-12	17.5	0.55
65	5-8	17.7	2.00
66	17-18	17.7	1.85
67	5	17.9	0.59
68	9-14	17.9	2.39
69[c]	5-8	18.0	1.51
70	9-11	18.2	1.04
71	18	18.2	0.46
72	5	18.4	0.32
73	11-13	18.4	0.45
74	5-9	18.5	1.81
75	15	18.5	0.71
76	18	18.5	0.25
77	13-15	18.7	0.92
78	16-18	18.7	0.94
79	9-13	19.1	2.30
80	14-16	19.3	1.61

---

[a] Source: Holmstead et al., 1974.

[b] Toxicant B - see Section 2.3.3.

[c] Toxicant A - see Section 2.3.3.

Table 2.4 (continued)

	Chromatographic properties		
Number	Silica gel fraction	Gc R_t, min	Amount in toxaphene (%)
81	17-18	19.3	0.88
82	9-10	19.5	0.92
83	12-13	19.5	0.43
84	17-18	19.7	0.88
85	7-8	19.8	0.83
86	11	19.8	0.33
87	13-14	19.9	0.68
88	6-9	20.1	1.79
89	10-13	20.3	1.41
90	15	20.3	0.46
91	18	20.3	0.74
92	15-16	20.7	1.00
93	18	20.7	0.54
94	13-14	21.1	0.17
95	18	21.1	0.69
96	12	21.3	0.09
$C_{10}H_9Cl_9$, $[M - Cl]^+ = m/e$ 409			
97	17	13.7	0.30
98	1	16.9	0.47
99	1	17.4	0.33
100	1-2	17.9	0.14
101	1-4	18.0	1.64
102	4	18.5	0.93
103	1	18.9	0.04
104	3-4	19.0	0.37
105	6-7	19.1	0.22
106	3	19.5	0.33
107	4-6	19.7	0.61
108	1-2	20.1	0.97
109	3	20.1	0.35
110	4-5	20.1	0.47
111	1	20.5	0.41
112	5-7	20.7	0.69
113	14	20.7	0.20
114	17	20.7	0.23
115	3-8	20.9	1.56
116	10-12	20.9	0.33
117	1-5	21.3	0.82
118	7-8	21.3	0.31
119	18	21.3	0.18
120	5	21.5	0.07

---

[a] Source: Holmstead et al., 1974.
[b] Toxicant B - see Section 2.3.3.
[c] Toxicant A - see Section 2.3.3.

Table 2.4 (continued)

Number	Chromatographic properties		Amount in toxaphene (%)
	Silica gel fraction	Gc R_t, min	
121	11	21.5	0.31
122	13-16	21.5	0.84
123	6-10	21.7	0.64
124	7-11	22.1	0.61
125	14-17	22.1	0.64
126	14	22.4	0.54
127	14-18	23.3	1.00
$C_{10}H_8Cl_{10}$, $[M - Cl]^+$ = *m/e* 443			
128	3-5	19.3	1.09
129	2-3	21.9	0.13
130	5	21.9	0.08
131	1-3	22.4	0.37
132	2-4	23.3	0.14
133	3	23.7	0.03
134	1-2	24.0	0.21
$C_{10}H_7Cl_{11}$, $[M - Cl]^+$ = *m/e* 477			
135	4	25.4	0.02
$C_{10}H_{10}Cl_6$, $[M - Cl]^+$ = *m/e* 305			
136	1-2	7.9	0.05
137	1	9.0	0.13
138	8	10.1	0.46
139	2	10.5	0.09
140	14	10.9	0.10
141	8-9	11.3	0.53
142	11-12	11.8	0.28
143	14	11.9	0.16
144	18	12.7	0.42
145	15	12.8	0.06
146	15-16	13.3	0.25
147	18	13.3	0.42
148	13	13.5	0.10
149	18	14.0	0.03
150	13	14.9	0.06
$C_{10}H_9Cl_7$, $[M - Cl]^+$ = *m/e* 339			
151	11	12.7	0.08
152	14	12.8	0.08
153	6	13.1	0.23
154	17	13.1	0.22
155	8-12	13.5	1.03

---

[a] Source: Holmstead et al., 1974.
[b] Toxicant B - see Section 2.3.3.
[c] Toxicant A - see Section 2.3.3.

Table 2.4 (continued)

Number	Chromatographic properties		Amount in toxaphene (%)
	Silica gel fraction	Gc R_t, min	
156	6	13.6	0.13
157	14	14.3	0.59
158	11-12	14.7	0.81
159	14	14.9	0.29
160	16	14.9	0.15
161	16	15.2	0.37
162	14-15	15.5	0.58
163	12-13	16.1	0.60
$C_{10}H_8Cl_8$, $[M - Cl]^+$ = m/e 373			
164	2-3	13.8	0.13
165	2	14.2	0.20
166	16-17	14.2	0.47
167	1-3	15.0	1.47
168	18	15.2	0.42
169	5	15.3	0.10
170	3-4	15.6	0.46
171	1	15.8	0.04
172	3	15.8	0.30
173	18	15.8	0.59
174	3	16.2	0.08
175	15	17.9	0.29
$C_{10}H_7Cl_9$, $[M - Cl]^+$ = m/e 407			
176	1	16.5	0.36
177	2	18.9	0.14

[a] Source: Holmstead et al., 1974.
[b] Toxicant B - see Section 2.3.3.
[c] Toxicant A - see Section 2.3.3.

molecular formula for each of the 177 individual constituents which they observed by GC. They have accounted for the observation of a constituent in two or more fractions, but have not accounted for the possibility that some of the peaks might have formed in the work-up or during GC. Isomers of $C_{10}H_{10}Cl_8$ and the combined isomers of $C_{10}H_{11}Cl_7$ and $C_{10}H_9Cl_9$ each accounted for approximately one-third of the total technical toxaphene. Most of the remaining constituents correspond to unsaturated or tricyclic isomers containing from six to eight chlorine atoms ($C_{10}H_{16-n}Cl_n$ where n is 6 to 8).

Black (1974) used the field ion mass spectrum of toxaphene to approximate the composition of components containing from five to eleven chlorine atoms. Table 2.5 summarizes the approximate composition of the polychlorinated species in toxaphene based on the chlorine isotope envelope.

Holmstead et al. (1974) reduced toxaphene with triphenyl tin hydride. They could only successfully identify bornane in the GC-MS of the reduction product. When Beckman and Berkenkotter (1963) used sodium-liquid anhydrous ammonia to reduce toxaphene, they suspected that α-pinene, camphene, β-pinene, d-limonene, and p-cymene were present in the reduced product by virtue of identical retention times in the GC patterns (Section 2.4.2).

2.4.2 Comparison of the Compositions of Toxaphene and Strobane

Since Strobane is a suspected carcinogen (Section 6.0), a comparison of its composition with the composition of toxaphene is important. Strobane, as well as other polychlorinated terpenes, is closely related in composition to toxaphene (Guyer et al., 1971; Melnikov, 1971). Although recent research has partly elucidated the composition of toxaphene, there has been no information from comparative studies with other polychlorinated terpenes. Thus, comparison of toxaphene and Strobane must rely solely on gross data from gas chromatographic analysis.

Table 2.5. Approximate composition of polychlorinated toxaphene constituents based upon field ion mass spectrum measurement[a]

Species	Ion current (%)
Cl-5	1.3
Cl-6	7.9
Cl-7	19.2
Cl-8	20.4
Cl-9	11.8
Cl-10	5.7
Cl-11	1.8
Total	68.1

[a] Source: Black, 1974.

Figure 2.3 compares their gas chromatograms under similar, but not identical, conditions. Black (1976) has confirmed that the GC profiles of Strobane and toxaphene are similar; they primarily differ in the relative peak heights of certain components.

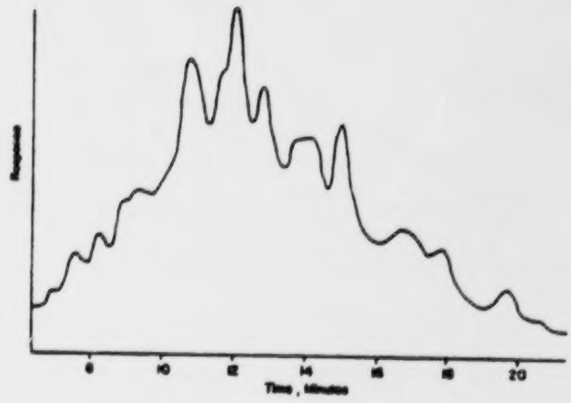
Beckman and Berkenkotter (1963) examined the reduction of Strobane and toxaphene with sodium-liquid ammonia. Both yielded similar gas chromatographs. Products, whose identities were assigned according to matching retention times, include α-pinene, camphene, β-pinene, d-limonene, and p-cymene. Relative GC peak heights of the reduction products from Strobane and toxaphene differed.

2.4.3 Fractionation and Isolation of Toxaphene Components

Several groups have used chromatographic methods to fractionate toxaphene and then have evaluated each fraction for differences in constituent composition, physical properties, and toxicological properties (Section 6.0). A few of the constituents have been isolated and structures have been assigned (Table 2.6). The constituents listed in Table 2.6 are considered the most toxic components.

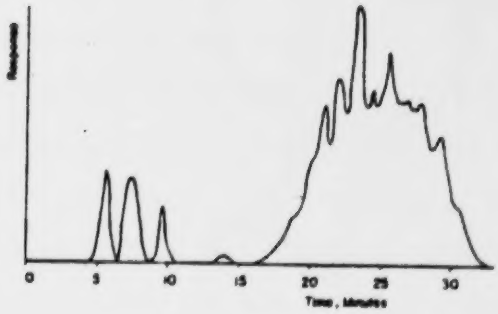
The constituent separations and individual component isolations have usually used combinations of chromatographic techniques. The procedures take advantage of differences in the separation of constituents by the different chromatographic techniques. For example, while gas chromatography and partition chromatography apparently separate constituents based upon chlorine content, column chromatography and thin-layer chromatography separate constituents based upon polarity (Holmstead et al., 1974).

Seiber et al. (1975) have challenged structural assignments by Black (1974) and Anagnostopoulos et al. (1974). Seiber et al. (1975) demonstrated that "non-polar 19" (Black, 1974) and Substance I (Anagnostopoulos et al., 1974) are identical since they have identical melting points and identical



Toxaphene response curves

Column, 30 feet, 30% SE-30 gum rubber on 30-60-mesh Chromosorb W, temp. 275°C, helium flow 70 cc/min.



Strobane response curves

Column, 2 feet, 20% SE-30 gum rubber on 30-60-mesh Chromosorb W, helium 55 cc/min, program rate 11°/min, initial temp. 50°C.

Figure 2.3. Comparison of the gas chromatographs of toxaphene and Strobane. Source: Beckman and Berkenkotter, 1963

Table 2.6. Structure and properties of constituents isolated from toxaphene

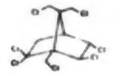
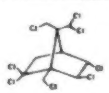
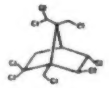
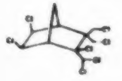
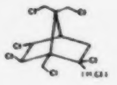
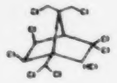
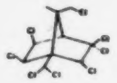
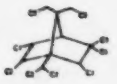
Name and synonyms	Molecular formula	Molecular structure	Melting point, C	Crystal structure	Verification of structure	Percentage in toxaphene	Sources
"Toxicant B" 2,5-endo,2,6-exo,8,9,10-heptachlorobornane	$C_{10}H_{11}Cl_7$		166-167	Orthorhombic (from 5:1 hexane-acetone)	N.M.R., x-ray crystallography	3%	Casida et al., 1974 Khalifa et al., 1974 Turner et al., 1975 Palmer et al., 1975
"Toxicant A" 2,5-endo,2,6-exo,8,8,9,10-octachlorobornane	$C_{10}H_{10}Cl_8$		134-136	Hexagonal (from isopropyl alcohol)	N.M.R.	6%	Casida et al., 1974 Khalifa et al., 1974 Turner et al., 1975 Nelson and Matsumura, 1975 a,b
and							
2,5-endo,2,6-exo,8,9,9,10-octachlorobornane	$C_{10}H_{10}Cl_8$						
2,5,6-exo,8,8,9,10-heptachlorodihydrocamphene	$C_{10}H_{11}Cl_7$		131-132	Parallelepiped	N.M.R.	0.8	Seiber et al., 1975 Landrum et al., 1976

Table 2.6 (continued)

Name and synonyms	Molecular formula	Molecular structure	Melting point, C	Crystal structure	Verification of structure	Percentage in toxaphene	Sources
Black, "component 13" 2,6-exo,3-endo,8,9,10-hexachlorobornane (tentative)	$C_{10}H_{12}Cl_6$ or $C_{10}H_{11}Cl_7$		215-220	Colorless needles (from carbon tetrachloride)	N.M.R.- Not certain		Black, 1974 Seiber et al., 1975
Black - "Non-polar 19" Anagnostopoulos - "Substance I" 2,3,5,6-exo,5-endo, or 2,3,5-exo,5,6-endo, 9,10,10-nonachlorobornane	$C_{10}H_9Cl_9$				N.M.R. Stereochemistry at C-6 is not certain		Black, 1974 Anagnostopoulos et al., 1974 Seiber et al., 1975
Anagnostopoulos - "Substance II" 2,3,5-exo,5,6-endo, 8,10,10-octachlorobornane	$C_{10}H_{10}Cl_8$				N.M.R.		Anagnostopoulos et al., 1974
Anagnostopoulos - "Substance III" 2,3,5,6-endo,5-exo, 8,9,10,10-nonachlorobornane	$C_{10}H_9Cl_9$				N.M.R.		Anagnostopoulos et al., 1974

GC properties. They could not conclusively identify the stereochemistry at C-5. Seiber et al. (1975) also suggest that "component 13" (Black, 1974) is a heptachlorobornane isomer similar in structure to Toxicant B, since their retention times are identical on three different columns. However, since the melting points differ, "component 13" and Toxicant B are not identical.

2.5 CHEMICAL REACTIVITY

Chemical reactivity of toxaphene has generally been examined for the mixture rather than by individual components. Some recent work on toxaphene reduction by Fe(II) examined reactivity of isolated constituents (Section 2.5.4). Except for this study (Khalifa et al., 1976), virtually no information is available on selective reactivity of toxaphene constituents.

Most of the information on toxaphene chemistry involves the lability of its carbon chlorine bonds (Hughes, 1970). The reactions include hydrolysis, substitution reactions (for example, with amines), elimination reactions, and reduction.

2.5.1 Hydrolysis

Mitchum (1963) measured the rates of loss of 1.0 mg/liter toxaphene in filtered, natural water at temperatures of 7, 18, and 26 C. The aqueous toxaphene was held in the dark, but not sealed. The extrapolated time for essentially complete degradation was 645 days at 26 C and 2,680 days at 7 C. Hughes (1970) has criticized the results since the initial toxaphene concentration exceeded apparent solubility. Hughes suggested that toxaphene loss might have resulted from evaporation.

Wolfe et al. (1976) have presented evidence which establishes that toxaphene is relatively stable to hydrolysis in both acidic and alkaline conditions. In their procedure, two solutions containing 1.3 ppm toxaphene in 5% aqueous acetonitrile were adjusted to pH 3.7 and pH 10.0 and then sealed in ampoules.

After the tubes were heated at 65 C for two days, the solutions were extracted with chloroform and the extracts were examined by GC. No differences were found between the GC patterns of the extracts and standard toxaphene.

2.5.2 Reactions Used for Analytical Clean-Up and to Prepare Derivatives of Toxaphene

The dehydrohalogenation of toxaphene by strong aqueous and alcoholic alkali has been used to prepare derivatives for GC analysis. Typical reaction conditions are presented in Section 2.6.4.1.2. The products have not been well characterized. Archer and Crosby (1966), who used ethanolic potassium hydroxide to simplify toxaphene's GC pattern, have partially characterized the product by infrared spectroscopy. This product exhibits a weaker absorbance in the 12 to 15 micron region, an increased absorbance in the carbon-hydrogen region at 3.5 microns, and a new carbonyl peak at 5.8 microns. These observations indicate a loss of carbon chlorine bonds along with a gain in olefin and some carbonyl production.

Several colorimetric analytical procedures utilize derivatives prepared by toxaphene reactions with amines. The most common derivative is prepared with diphenylamine (Graupner and Dunn, 1960; Faucheux, 1965; Adamovic, 1968; Klein and Link, 1970; Thielemann, 1973). The procedures use different techniques, e.g., Lewis acid catalysts or heat, to accelerate the reaction. Some reaction conditions are discussed in Section 2.6.4.3. The reaction products have not been characterized.

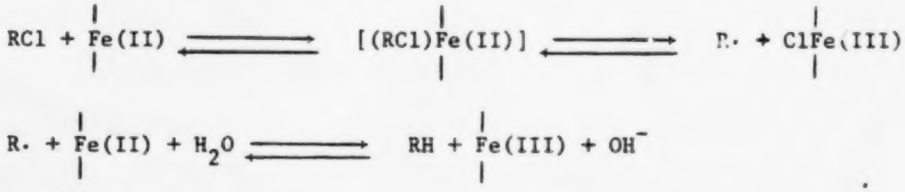
Toxaphene is stable in strong Bronstead acids. The stability has been applied to several clean-up procedures: equal volume mixtures of sulfuric and fuming sulfuric acid (Mills, 1959); sulfuric and fuming nitric acid (Klein and Link, 1970; Kawano et al., 1969); and acetic and perchloric acid (Hughes, 1970) (Section 2.6.3).

2.5.3 Oxidation-Reduction

Metals and metal ions can reductively dechlorinate toxaphene. The only identified reduction products are chloride ion and less chlorinated terpenes (Section 2.4.1) (Beckman and Berkenkotter, 1963; Turner et al., 1975).

Toxaphene is more easily reduced by zinc and acetic acid than chlordane, dieldrin, heptachlor, endrin, or aldrin (Hornstein, 1957; Sweeny and Fischer, 1970). Sweeny and Fischer (1970) examined chloride released by the reaction of 1 g of chlorinated hydrocarbon with 1 g of zinc powder in 20 ml of acetone and 10 ml of 10 percent aqueous acetic acid. While toxaphene released 61.6 percent of its theoretical chloride, chloride release from the other chlorinated hydrocarbons ranged from 19.0 to 29.6 percent of theoretical.

Khalifa et al. (1976) have partially elucidated the chemistry by which iron in biological systems reduces toxaphene. They used standard toxaphene and two isolated components, Toxicants A and B (see Table 2.6 for molecular and structural formulas). Two sources of iron complexed with protoporphyrin were examined: from hematin and from liver microsomes. They demonstrated that toxaphene degradation proceeds by dehydrochlorination or reductive dechlorination and that Fe(II) is the reducing agent. They proposed that the reductive dechlorination proceeds by the following sequence:



$$RCl + \overset{|}{\underset{|}{Fe}}(II) \rightleftharpoons [(RCl)\overset{|}{\underset{|}{Fe}}(II)] \rightleftharpoons R\cdot + Cl\overset{|}{\underset{|}{Fe}}(III)$$

$$R\cdot + \overset{|}{\underset{|}{Fe}}(II) + H_2O \rightleftharpoons RH + \overset{|}{\underset{|}{Fe}}(III) + OH^-$$

Regeneration of Fe(II) required a reducing agent; they used $Na_2S_2O_4$ with hematin and NADPH (nicotinamide adenine dinucleotide phosphate) with microsomes. Neither $Na_2S_2O_4$ or NADPH reduced toxaphene in the absence of iron protoporphyrin.

Khalifa et al. (1976) partially delineated the reaction products from Toxicants A and B. Since the reaction products exhibited relatively high R_f values (TLC on silica gel), they suggested that the products are more polar than the original toxaphene constituents. They used GC-MS to identify the molecular formulas of the primary products from reaction with the hematin-$Na_2S_2O_4$ system. Toxicant A ($C_{10}H_{10}Cl_8$) yielded two reductive dechlorination products ($C_{10}H_9Cl_7$) and two products with molecular formula $C_{10}H_{10}C_6$. Toxicant B ($C_{10}H_{11}Cl_7$) formed two reductive dechlorination products ($C_{10}H_{12}Cl_6$), one dehydrochlorination product ($C_{10}H_{10}Cl_6$) and two products with the molecular formula $C_{10}H_{11}Cl_5$.

2.5.4 Photochemistry

Toxaphene appears to be less reactive in response to sunlight irradiation than in response to irradiation by higher energy light. Available studies have examined its photochemistry in solution and as a spot on chromatographic paper.

Mitchell (1961) irradiated 141 pesticide chemicals including toxaphene (spotted on chromatographic paper) at 253.7 nm. His results suggest that toxaphene is degraded more slowly than aromatic organochlorides.

Friedman and Lombardo (1975) irradiated toxaphene (9 to 10 micrograms in petroleum ether) at 253.7 nm for 90 minutes. Their interest was to eliminate interferences in the analysis of chlorinated paraffins and not to evaluate the stability of toxaphene. They observed that after the above irradiation approximately 36 percent of the toxaphene peak area (GC) remained. Since sunlight cuts off at 280 nm (Wolfe et al., 1976), toxaphene may be more stable under environmental conditions.

Wolfe et al. (1976) have examined the effect of simulated sunlight irradiation (cut-off was at 280 nm) of a saturated solution of toxaphene in filtered, natural water. They reported that toxaphene degraded slowly, but included no further information on rates or products. They also reported that toxaphene is not readily oxidized by photochemically-generated (methylene blue sensitizer) singlet oxygen in aqueous solution.

2.6 ANALYSIS

2.6.1 Considerations in Analysis

Toxaphene analysis follows the same general procedure as the analysis of other organochloride residues. Several references have specifically reviewed the important procedures for toxaphene analysis (Hughes, 1970; Guyer et al., 1971; Hartwell et al., 1974).

Prior to 1963, gas chromatography was not used for toxaphene analysis. The most popular methods used then were total chloride determinations and colorimetric methods (Hartwell et al., 1974). These methods were not as sensitive as GC and their specificity was low in some cases (e.g., total chloride methods measured all chlorine present in the sample).

Gas chromatographic analysis has become the method of choice for measuring toxaphene. Since numerous organic materials can interfere with GC analysis, the experimental procedures reported in the literature should be carefully examined to insure that interferences were removed before analysis.

2.6.2 Sample Collection and Extraction

Samples taken for toxaphene analysis follow the same general rules as samples taken for other organochloride residues. The sample should be representative of the source and the collection and storage should prevent toxaphene loss (e.g., vaporization or degradation) or contamination by interfering substances (Taras et al., 1971; Thompson et al., 1974). Since containers should not introduce interferences (especially plasticizers and plastic additives), all-glass containers and equipment should be used. Thompson et al. (1974)

recommends that environmental samples should either be extracted within 24 hours or frozen (-12 to -18 C).

2.6.2.1 Air -- Air has been sampled for low toxaphene residue concentrations using high volume samplers (Stanley et al., 1971; Arthur et al., 1976; Bidleman and Olney, 1974, 1975; Bidleman et al., 1976). Stanley et al. (1971) used a combination of glass cloth filtering, impingement (Greenburg-Smith impinger with hexylene glycol as the trapping solvent), and adsorption on alumina. Their extraction procedure used methods that were developed for measuring food residues. Arthur et al. (1976) used a MISCO model 88 air sampler with ethylene glycol as the trapping solvent. Bidleman (1974, 1975, 1976) used a combination of a glass fiber filter and a polyurethane foam plug. Residues were desorbed by Soxhlet extraction with petroleum ether.

2.6.2.2 Water -- Water can be collected as grab samples or sampled by adsorption methods, usually with activated carbon. The advantage of adsorption is that concentrated residues from large volumes of water are obtained; its disadvantages include degradation on the column and poor recovery (Hinden, 1964; Morley, 1966; Faust and Suffet, 1966).

Perhaps typical of the liquid-liquid extraction procedure is the method used by Schafer et al. (1969). They collected 3.5 liter water samples in narrow-necked, gallon glass jugs. Organochlorides were extracted by adding 10 ml of hexane to the jug and agitating with magnetic stirring. The hexane layer was removed by filling the jug with water until the hexane reached the conical neck; the hexane layer was then removed by pipette. With 2.5 ppb toxaphene samples, their recovery was 94.7 ± 11.5 percent.

One of the more common adsorption procedures is the carbon-chloroform extraction (CCE) (Nicholson et al., 1964). During CCE procedure, a known volume of water is pumped through a carbon cylinder and the toxaphene is sequentially desorbed by Soxhlet extraction with chloroform. Nicholson et al. (1964) reported that toxaphene recovery from spiked samples averaged less than 50 percent; at 1.0 ppb, average recovery was 48 percent (range 42 to 51 percent) and at 0.5 ppb, it averaged 42 percent (39 to 47 percent).

2.6.2.3 Soils and Sediments -- Soils, suspended solids, and bottom sediments are collected and extracted by similar procedures. Several workers (Woolson and Kearney, 1969; Williams, 1968; Nash and Harris, 1972; Saha et al., 1969; Saha, 1971) have evaluated the recovery of organochloride pesticides other than toxaphene by several different extracting methods and with varying amounts of soil moisture. They concur that recovery by all extraction methods is better from moist (20 percent water), rather than dry, soils. Saha (1971) considers hexane-acetone and hexane-isopropyl alcohol azeotropes the solvents of choice. Saha et al. (1969) reported that recoveries of selected organochlorides with hexane-isopropyl alcohol extraction by Soxhlet extractor, mechanical shaking, ultrasonic vibration, ball mill, and blending ranged from 90 to 97 percent for soil containing 20 percent water.

Mechanical shaking and Soxhlet methods appear the most popular methods for extracting soils and sediments for toxaphene. Veith and Lee (1971) used Soxhlet extraction with a hexane-acetone azeotrope for sediments. Their recovery for spiked samples with 30 hour extraction time was 88.3 ± 2.9 percent. Parr and Smith (1976) used the same conditions for extracting moist soil. Toxaphene recovery from spiked samples extracted from 24 to 48 hours approached 100 percent. Nash and Woolson (1967, 1968) extracted toxaphene and other organochloride insecticides from moist soils by mechanical shaking of the sample with hexane-isopropyl alcohol (3:1 by volume). While no recoveries were reported for toxaphene, they did recover other organochlorides at 85 to 100 percent. Nash et al. (1973) used the Soxhlet method, mechanical shaking, and column extraction to extract toxaphene and other organochlorides from moist soils. Solvents were as follows: for Soxhlet extraction, hexane-acetone-methanol (8:1:1); for mechanical shaking, hexane-acetone azeotrope; and for column extraction, hexane: acetone: methanol (4:3:3). While they reported extraction efficiencies to be best by shaking and poorest by column extraction, they noted that differences were not statistically significant. Goerlitz and Law (1974) reported a mechanical shaking technique for extracting toxaphene and other organochloride pesticides. The sample (50.0 g) is first agitated with acetone (40 ml); then hexane (80 ml) is added and agitation is resumed. The solvent is decanted and the procedure repeated. While they did not examine recovery of spiked toxaphene samples, recovery of other organochlorides ranged from 75 to 99 percent with an average of 97.9 percent.

2.6.2.4 Biological Specimens -- Plant and animal tissues, food, milk, blood, and other biological samples are collected and pretreated similarly to soils and water samples. Tissues should be stored in screw-cap jars and blood in screw-cap vials. Tissue samples which are to be extracted within 24 hours can be held at refrigerator temperature (+2 to +4 C), but if they are to be held longer before extraction, they should be kept at -18 to -12 C (freezer temperature). Blood samples should be centrifuged as soon as possible after drawing. The serum may be held at +2 to +4 C for up to 3 days or at -18 to -12 C for longer periods (Thompson et al., 1974). Better recovery is reported if sulfuric acid is added to the whole blood before centrifuging (Stretz and Stahr, 1973; Griffith and Blanke, 1974). Tissues are homogenized in a food chopper or Waring blender, then subsequently extracted with a hydrocarbon solvent such as hexane, either during the blending or by shaking. Samples with high lipid content require a partitioning (e.g., hexane/acetonitrile partitioning) to reduce the lipids to a low enough level for clean-up by sulfonation and chromatography (Section 2.6.3). Samples with low lipid concentration can begin clean-up at the sulfonation and chromatography steps. The hexane (or other hydrocarbon) extract is cleaned up and pretreated similarly to extracts from other sources (Mills, 1959; Giuffrida et al., 1966; Samuel and Hodges, 1967; Hughes, 1970; Burke, 1971; Thompson et al., 1974).

2.6.3 Clean-Up and Pretreatment

Because toxaphene is a complex mixture and numerous organochlorides can interfere with its analysis, work-up procedures are more important to its analysis than for organochloride pesticides of simpler composition, such as DDT, aldrin, heptachlor, etc. Work-up procedures must remove the naturally occurring organic products and synthetic organochlorides which will interfere with the analysis. The procedures use differences between toxaphene and the interferences in terms of solubility properties, adsorption characteristics, and chemical reactivity.

Naturally occurring waxes, fats, oils, and pigments are removed by partitioning, by sulfonation, and/or by column chromatography. Partitioning is generally used for samples with high fat content (tissues, foods, etc.) before acid treatment or column chromatography. The method developed by Mills (1959) has been used for toxaphene as well as the other organochloride pesticides. In the technique, toxaphene and the naturally occurring lipids are partitioned between hexane and acetonitrile; the organochlorides are dissolved in the acetonitrile and the lipids in the hexane. Mills (1959) recommends that 15 ml or more of hexane be used per gram of fat. If less hexane is used, the dissolved fat-hexane layer becomes more soluble for toxaphene and yields a low residue measurement.

After high lipid concentrations are reduced by the partitioning procedure, residual lipid concentrations can be removed by sulfonation and chromatography; the sulfonated products are held up on the column. This can be accomplished either by treating the sample extract in a hydrocarbon solvent (hexane, petroleum ether, etc.) with a sulfuric-fuming sulfuric acid mixture or by passing the extract through an acid-Celite column, which is prepared from 5 grams Celite 545, 1.5 ml of concentrated sulfuric acid, and 1.5 ml of 15 percent fuming sulfuric acid (Hartwell et al., 1974).

While sulfonation removes the naturally occurring interferences, it does not remove other organochlorides. The stronger conditions of sulfuric acid-fuming nitric acid treatment do remove some of the organochlorides (e.g., DDT and methoxychlor), as well as the natural products (Erro, 1967; Guyer et al., 1971; Hartwell et al., 1974). In procedures developed by Kawano et al. (1969) and Klein and Link (1970), the sample (ca. 50 to 100 micrograms of toxaphene) was dried and the residue was dissolved in 5 ml of ice cold sulfuric-fuming nitric acid. The acid solution was diluted with 150 ml of distilled water and ice and extracted with petroleum ether (three times). Nitrated DDT and other organochloride derivatives can then be removed by chromatographic methods. However, this procedure will not remove polychlorinated biphenyls (PCB's). Kowano et al. (1969) reports that toxaphene recovery from this treatment ranges from 79 to 96 percent with a mean of 92 percent.

Archer and Crosby (1966) used alkaline dehydrohalogenation to clean up organochloride interferences (e.g., DDT is converted to DDE which can be separated from toxaphene by column chromatography) and to simplify the toxaphene GC pattern (Section 2.6.4.1.2). They reacted the toxaphene with 5 g of potassium hydroxide dissolved in 20 ml of aqueous alcohol (3 ml of water and 17 ml of ethanol).

Column chromatography (CC) will remove trace residues of natural products and will fractionate organochlorides. The fractionation can divide organochlorides into smaller groups or can remove potential interferences, such as PCB's. Mills et al. (1963) developed the standard technique for fractionating food residues by CC on Florisil using solvents of 6 percent and 15 percent ethyl ether in petroleum ether; toxaphene elutes with the 6 percent ethyl ether fraction. Mills et al. (1972) have developed a three solvent system for CC on Florisil: (A) 20 percent methylene chloride and 80 percent hexane; (B) 50 percent methylene chloride, 0.35 percent acetonitrile, and 49.65 percent hexane; and (C) 35 percent methylene chloride, 1.5 percent acetonitrile, and 48.5 percent hexane. Solvent A elutes toxaphene. In both the above procedures, PCB (as well as other interfering organochlorides) will elute with the toxaphene.

Several CC procedures are available which separate toxaphene from PCB's. Reynolds (1969) found that PCB, heptachlor, aldrin, and DDE are eluted with hexane while toxaphene, along with heptachlor epoxide, dieldrin, DDT, and DDE, will elute later with 20 percent ethyl ether in hexane. Armour and Burke (1970) reported that a silicic acid/Celite 545 column, which was prepared from 5 g acid-washed Celite and 20 g of activated silicic acid, separated PCB from toxaphene. When they chromatographed the 6 percent ethyl ether/petroleum ether fraction from a Florisil column, PCB eluted with petroleum ether while toxaphene was eluted later with acetonitrile-hexane-methylene chloride (1:19:80). While they did not examine toxaphene recovery, they did recover nine other organochlorides by this technique at 80 to 107 percent.

Hughes et al. (1970) used columns packed with multiple adsorbent layers for toxaphene clean-up. The columns which were used to clean up extracts of water and suspended solid samples were packed in three layers; Florisil; 1:1 mixture of magnesium oxide and Celite; and then anhydrous sodium sulfate. The chromatography column for clean-up of plankton extract also contained three layers: 4:1 mixture of Florisil and Celite; alumina (+ 1 percent water); and anhydrous sodium sulfate. The former column was eluted with 6 percent ethyl ether in hexane and the latter with 10 percent ethyl ether in hexane. The MgO converts DDT to DDE, which elutes before toxaphene, but the MgO does not alter toxaphene.

Goerlitz and Law (1974) used a two column procedure to separate organochlorides in sediment extracts. They chromatographed 0.5 ml hexane extracts on 8 cm long (10 mm id) alumina and silica gel columns and collected two fractions on both. The alumina column yielded two fractions: A-1 (0-20 ml hexane), which contained toxaphene, aldrin, chlordan, DDT and its residues, heptachlor, lindane, PCB, and polychlorinated napthalenes (PCN's) and A-2 (20-35 ml hexane), which contained dieldrin, endrin, and heptachlor epoxide. Fraction A-1 was chromatographed on silica gel and yielded fraction S-1 (0-25 ml with hexane), which contained aldrin, PCB, and PCN and S-2 (25-45 ml with benzene), which contained toxaphene and the remaining organochlorides. Although they did not report toxaphene recoveries, they obtained 75 to 99 percent recovery with fortified samples of nine other organochloride insecticides.

Aquatic sediment samples contain numerous organosulfur compounds which might not be removed by chromatographic methods. Goerlitz and Law (1971) report that treating the sediment extract with metallic mercury prior to column chromatography removes these potential interferences.

2.6.4 Toxaphene Measurement

Table 2.7 lists analytical methods for toxaphene with approximate lower detection limits. Table 2.8 compares sensitivity, recovery, and variability for some toxaphene analysis procedures. Since gas chromatography appears to be the most widely used procedure, their parameters are of special interest. Detection by microcoulometry and electron capture exhibit similar sensitivities (Burke and Holswade, 1966). Toxaphene at environmental concentrations (ca. 1 ppm) generally exceeded 70 percent recovery.

The analysis for toxaphene in ambient, environmental samples proceeds as follows: toxaphene must be identified; the identification confirmed; and the quantity measured. The usual procedure for pesticide analysis in ambient samples consists of clean-up and then screening by a chromatographic method (Burke, 1971). Gas chromatography (GC) is the preferred method, but other chromatographic forms, in particular thin-layer chromatography (TLC), are useful, less expensive alternatives (Ismail and Bonner, 1974). Gas chromatography identification does require confirmation. Suggested methods include other chromatographic methods (TLC or reverse phase thin-layer chromatography) and GC with columns of different polarity. However, an independent measurement is preferred. Gas chromatography using chemical ionization-mass spectrometric detection is a relatively recent advancement which provides specific analysis for toxaphene at a low detection limit. However, capital and operating costs are substantially higher than other detection methods.

While colorimetry is still sometimes used to measure toxaphene in ambient samples, infrared spectrometry and total chloride measurements are no longer used. Total chloride, although sensitive to 100 ng, will measure all chloride. Unless the sample is known to contain no other source of chloride, the method

Table 2.7. Analytical methods for toxaphene[a]

Method	Approximate lower limit of detection	Specific for
Gas chromatography		
Electron capture detection	5 ng[b]	All organochlorides and other organics
Microcoulometric detection	10 ng[c]	All organochlorides
Specific ion mass spectrometry	5 ng	Specific for toxaphene but some interferences might be detected (e.g., Strobane)
Paper chromatography	200 ng	Other organics
Thin-layer chromatography	500 ng	Other organics
Colorimetric (Diphenylamine)	5 μg	Other organochlorides
Infrared spectrometry	10 μg	Other organochlorides and other organics
Total chloride analysis	100 μg	All sources of chloride

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[a] Source: Burke and Holswade, 1966; Griffith and Blanke, 1974; Faucheux, 1965; Ismail and Bonner, 1974; Widmark, 1971; Thurston, 1976.

[b] 25 to 30 ng for ½ Full Scale Deflection.

[c] 30 ng for ½ Full Scale Deflection.

Table 2.8. Measurements of variability, recovery, and sensitivity for several toxaphene analytical procedures

Method	Sample	Recovery (%)	Variability	Sensitivity	Reference
G.C. - M.C.D.[a]	Standard toxaphene			22 ng (1/2 FSD)	Burke and Holswade, 1966
G.C. - E.C.D.[b]	Standard toxaphene			25-30 ng (1/2 FSD)	Burke and Holswade, 1966
G.C. - E.C.D. with sulfuric acid - fuming nitric acid clean-up	50 ng 100 ng 200 ng	86-96% 92-96% 79-97%			Kawano et al., 1969
G.C. - E.C.D. with Mills, Only, Braither work-up	0.1 ppm in milk 0.75 ppm in corn oil 0.75 ppm in butterfat	80.5 90.0 90.0			Giuffrida et al., 1966
G.C. - M.C.D. - extraction by sulfuric acid method	100 to 500 ng added to 2 ml whole blood	100%			Griffith and Blanke, 1974
G.C. - E.C.D. - extraction into hexane	2.5 ppb in drinking water	94.7 ± 11.5	± 11.2%		Schafer et al., 1969
G.C. - E.C.D. - column chromatography clean-up	0.3 ng in natural water 1.0 ng in natural water 2.0 ng in natural water	97 100 101	± 0.22 ng (six determinations) ± 0.052 ng (six determinations) ± 0.071 ng (six determinations)		Hughes et al., 1970
Spectrochemical diphenylamine with column clean-up or sulfuric acid methylene chloride extraction	From 0 to 1000 ppm in grain extracts 0 to 1000 in blank runs	69-100 98 + 10	1-12% ± 7%		Graupner and Dunn, 1960
Combustion and total chloride analysis	1 to 50 μg		+ 1 to 4μ		Hudy and Dunn, 1957
I.R.			0.5% (absolute)		Clark, 1962

[a] G.C. - M.C.D. = Gas chromatography with micro-coulometric detection.

[b] G.C. - E.C.D. = Gas chromatography with electron capture detection.

cannot be used. Infrared analyses have been used for product assay but are not useful for ambient samples (Guyer et al., 1971; Hartwell et al., 1974).

2.6.4.1 Gas Chromatography -- Gas chromatography is the best developed method for both identifying and measuring toxaphene residues (Widmark, 1971; Hartwell et al., 1974). With mass spectral (MS), electron capture, or microcoulometric detectors, GC is capable of measuring residues at the ppt range. Table 2.9 lists some of the columns on which toxaphene retention times have been characterized.

The major problems in GC analysis result from the complexity of toxaphene's pattern, the pattern changes caused by environmental weathering, and the numerous interferences with retention times within the range of the toxaphene pattern (Erro et al., 1967). Table 2.10 lists relative retention times and response factors (electron capture detector) for potential interfering residues (Burke and Holswade, 1966). The problems which result from the complex GC pattern and numerous potential interferences are alleviated by specific clean-up procedures and by use of key peaks for quantitative analysis. The following sections focus on the effects of environmental weathering, the methods for optimum analysis by simplifying the GC pattern, and specific ion detection methods.

2.6.4.1.1 Effect of environmental weathering upon the GC peak pattern -- Environmental weathering selectively transforms and degrades toxaphene constituents by chemical, biological, and physical means. Gas chromatographic examination of weathered toxaphene has established that peaks at long retention times become reduced in size while new peaks appear at shorter retention times (Hughes, 1970). Different environmental conditions yield residues with differing, although recognizable, toxaphene patterns (Hughes, 1970). Bioassay of these samples showed that the toxaphene had lost its insecticidal activity.

Table 2.9. Gas chromatographic columns used for toxaphene analysis

Source	Column	Relative retention (aldrin = 1.00) of characteristic peaks
Bevenue and Beckman (1966)	5% QF-1 on Chrom W	6.0, 7.0, 8.0, and others
Windham (1969)	8% QF-1 + 2% OV-17 on Gas Chrom Q	4.34 and others
Burke and Holswade (1966)	(1:1) 15% QF-1 on Gas Chrom Q and 10% DC-200 on Gas Chrom Q	See Table 2.10
Burke and Giuffrida (1964)	10% DC-200 on Anakrom ABS	1.18, 1.51, 1.76, 2.63, 4.31, 4.51 and others
Archer and Crosby (1966)	5% Dow-710 + 5% SE-30 on Chrom W	See Figure 2.4
Hartwell et al. (1974)	3.8% UCW-98 on Diataport S	

Table 2.10. Relative retention times of pesticides and related compounds and their electron capture detection response[a,b,c]

Pesticide	Retention time relative to aldrin	Response ng for ½FSD
DDT (tech.)	0.39, 1.88, 2.48, 2.70, 3.28	3.5
Methoxychlor	0.51, 3.23, 4.8	10
Kelthane (tech.)	0.58, 0.74, 1.09, 1.31, 1.86, 2.21, 2.37	6
δ-BHC	0.68	0.5
Heptachlor	0.81	0.5
Limite	0.82, 1.31	15-20
Isopropyl ester 2,4,5-T	0.84	2
Dichlone	0.86, 2.04	4
Dimethoate	0.97	8
Ronnel	0.97	1
Aldrin	1.00	0.6
Telodrin	1.14	0.8
o,p'-TDE olefin	1.21	10
Isodrin	1.25	0.8
Perthane olefin	1.31	40
p,p'-Kelthane	1.31	5
Chlorobenzilate	1.33, 2.93	100-200
Methyl parathion	1.42	2.5
o,p'-DDE	1.46	2
Heptachlor epoxide	1.47	1
Malathion	1.48	20-30
p,p'-TDE olefin	1.50	7
Chlorbenside	1.51	1.8
Dacthal	1.52	1.5
α-Chlordane	1.57	2
β-Chlordane	1.73	1.2
p,p'-DDE	1.88	1.5
Parathion	1.88	5
Thiodan	1.89, 2.92	2-3
o,p'-TDE	2.04	1.5
Captan	2.10	4

[a] Source: Burke and Holswade (1966).

[b] Major peaks underlined.

[c] The following conditions were used with the gas chromatograph: column: mixture of equal portions 15% QF-1 (10,000 cst) on 80/100 mesh Gas Chrom Q and 10% DC-200 (12,500 cst) on 80/100 Gas Chrom Q, glass, 6 ft x 4 mm i.d.; column temp. 200°C, injection temp., 225°C, gas flow rate, 120 ml N_2/min; detector: electron capture, concentric-type, tritium source; detector voltage: DC voltage at which 1 ng heptachlor epoxide caused ½ full scale recorder deflection at 1 x 10^9 AFS sensitivity, attenuator, 1, recorder, 5 mv.

Table 2.10 (continued)

Pesticide	Retention time relative to aldrin	Response ng for ½FSD
Dieldrin	2.22	1-1.5
Paraoxon	2.25	15-20
o,p'-DDT	2.48	3
Endrin	2.55	2.5-3
Sulphenone	2.62	3-4
Kepone	2.67	7
p,p'-TDE	2.70	1.5
Ovex	2.78	3-4
p,p'-Methoxychlor olefin	3.00	10-12
Ethion	3.23	10
p,p'-DDT	3.28	3
Trithion	3.33	4
Endrin alcohol	3.45	4
Endrin aldehyde	3.98	4.5
p,p'-Methoxychlor	4.80	10
Prolan	4.90	10-15
Dilan	4.40, 4.98, 5.70	15
Mirex	5.15	6
Bulan	5.70	5-10
Delta Keto 153 (endrin product)	6.05	7
Imidan	7.54	10
Tedion	8.75	8-10
Guthion	9.3	50-100
Co-Ral	23.5	50-75
Chlordane (tech.)	0.72, 0.81, 1.07, 1.55, 1.72, 2.78	7
Toxaphene	1.70, 2.25, 2.37, 2.55, 3.03, 3.66, 4.10, 4.70, 5.8	25-30
Strobane	1.09, 1.38, 1.53, 1.69, 2.16, 2.52, 3.01, 3.56, 4.57	40

2.6.4.1.2 Attempts to simplify the toxaphene GC pattern -- Three methods of simplifying the toxaphene analysis by GC have been evaluated: peaks can be condensed by use of short columns; the number of peaks can be reduced by chemically making derivatives and quantitation can use a few characteristic peaks instead of the whole pattern.

Witt et al. (1962) reduced the complex toxaphene pattern into a single peak by using a 1 1/4 foot column instead of a conventional 6 foot column. They reported a retention time of less than 2 minutes and detection to 500 ng (microcoulometry). The method, however, is not usually feasible when other residues with similar retention times are present, because those peaks would overlap (Section 2.6.4.1.3).

Several groups evaluated quantitation by the use of a few key peaks of the complex toxaphene patterns. Gaul (1966) recommended toxaphene measurement by planimetry of the four final peaks of a chromatogram using 10 percent DC-200. The procedure is accurate to two significant figures and yields similar values to calculations using the whole toxaphene pattern. Gaul (1966) pointed out, however, that GC patterns for weathered residues will differ from standard toxaphene and results might differ between the two methods. DDT and methoxychlor can influence baseline construction for the final four peaks, but their effect can be corrected. Bevenue and Beckman (1966) recommended calculations use the three peak fingerprints of chromatograms obtained in a 5 foot column of 5 percent QF-1 on Chromosorb W. The toxaphene fingerprint elutes at relative retention times (aldrin = 1.00) of 6.0, 7.0, and 8.0, while p,p'-DDT elutes at 4.1. Klein and Link (1967) reported using various individual peaks for estimating toxaphene.

Erro et al. (1967) have criticized exclusive reliance on a fingerprint. They report that the last four peaks might not be present, which they attribute to weathering of the sample and/or the condition of the GC column. They concluded that removal of the interferences is the surest procedure and suggested that all samples which are to be analyzed for toxaphene should be treated with sulfuric-fuming nitric acid.

Archer and Crosby (1966) recommended alkaline dehydrohalogenation of samples containing toxaphene. Figure 2.4 illustrates the effect of dehydrohalogenation on the chromatogram of toxaphene on a 9 foot column packed with 5 percent DC-710 and 5 percent SE-30 on Chromosorb W at 200 C and nitrogen flow of 40 to 60 ml/min. The major toxaphene peak (3.50 minutes) elutes before DDT residues (6.25 min).

2.6.4.1.3 Gas chromatography - Specific ion monitoring--The best method for toxaphene analysis is gas chromatography with specific ion monitoring (SIM). The technique has been developed to yield a Limited Mass Reconstructed Gas Chromatogram (LMRGC) virtually specific for toxaphene; it is capable of detecting less than 5 ng (Thurston, 1976).

The method requires chemical ionization mass spectrometry (CI-MS), since the CI mode yields better resolution for the major fragments such as [M-Cl], [M-Cl-HCl], and [M-Cl-2HCl] ions. Electron impact mass spectrometry is not sufficiently sensitive (Thurston, 1976; Holmstead et al., 1974; Stalling and Huckins, 1975). The technique has been developed for the Finnigan 1015 quadruple mass spectrum with System 150 data system. The programs are written for PDP 8 (Thurston, 1976; Neher and Hoyland, 1974; Budde, 1976; Eichelberger et al., 1974).

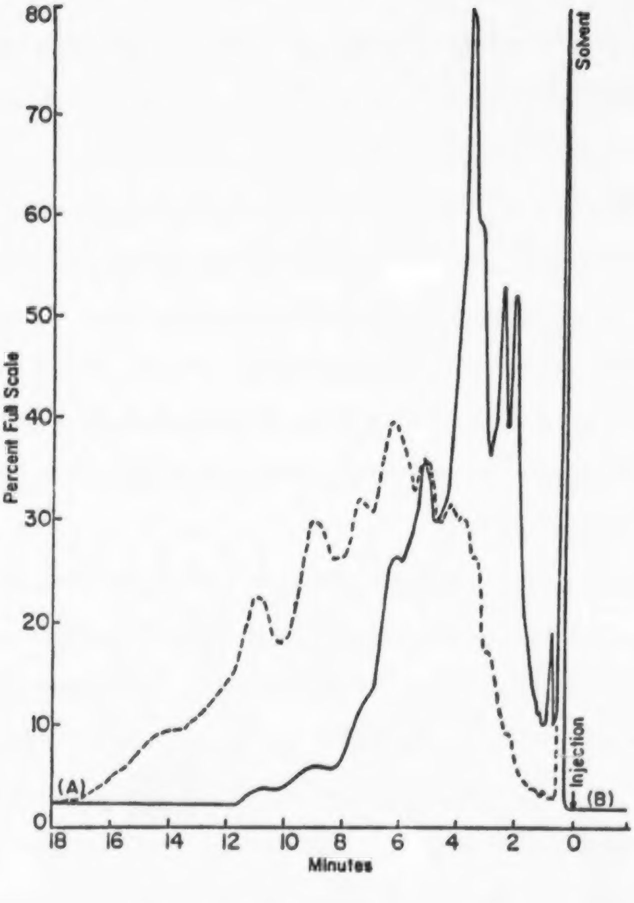


Figure 2.4. Gas chromatogram from 30 ng of toxaphene (A) before alkali treatment and after alkali treatment (B). Source: Archer and Crosby, 1966.

Thurston (1976) has developed LMRGC for a 60 cm x 2 mm (I.D.) glass column packed with 3 percent SP 2100 on 80/100 Supelcon AW. A short column can be used since detection is specific for toxaphene. The m/e 307 and 343 ions are judged as the optimum fragments for monitoring toxaphene (Thurston, 1976). Tetrachlorobiphenyl monitored at m/e 291 or 295 is used as internal standard. Figure 2.5 compares the reconstructed gas chromatogram (RGC) and LMRGC for a 10:1:1:1 mixture of toxaphene, DDE, TDE, and DDT to the LMRGC for toxaphene. Toxaphene is obscured in the RGC (reconstructed gas chromatogram), but well-defined by its LMRGC. LMRGC also specifically detects toxaphene with Arochlor 1260 present. Figure 2.6 compares LMRGC for toxaphene added to New Orleans drinking water in which 80 organic chemicals were found in concentrations of 0.05 to 10 micrograms per liter.

2.6.4.2 Other Chromatographic Methods--Paper chromatography, thin-layer chromatography (TLC), and reverse phase thin-layer chromatography (RPTLC) are less expensive although less sensitive analytical methods than GC. They can be used to screen samples, to confirm residues identified by another method, or for quantitative purposes [optical measurements are recommended for quantitative analysis (Getz, 1971)].

Since toxaphene chromatographs as streaks in TLC methods just as it does in GC, potential interferences must also be eliminated (Bontoyan and Jung, 1972; Walker and Beroza, 1963; Ismail and Bonner, 1974). Alkaline treatment to simplify chromatographic patterns has not been evaluated for chromatography other than GC.

Hartwell et al. (1974) and Guyer et al. (1971) recommend TLC on alumina oxide treated with silver nitrate using hexane as the solvent. The technique, as developed by Schechter (1963) and Moats (1966), has a limit of detection to ca. 500 ng for toxaphene, which appears as a streak. The color is developed by exposing the plate to ultraviolet light.

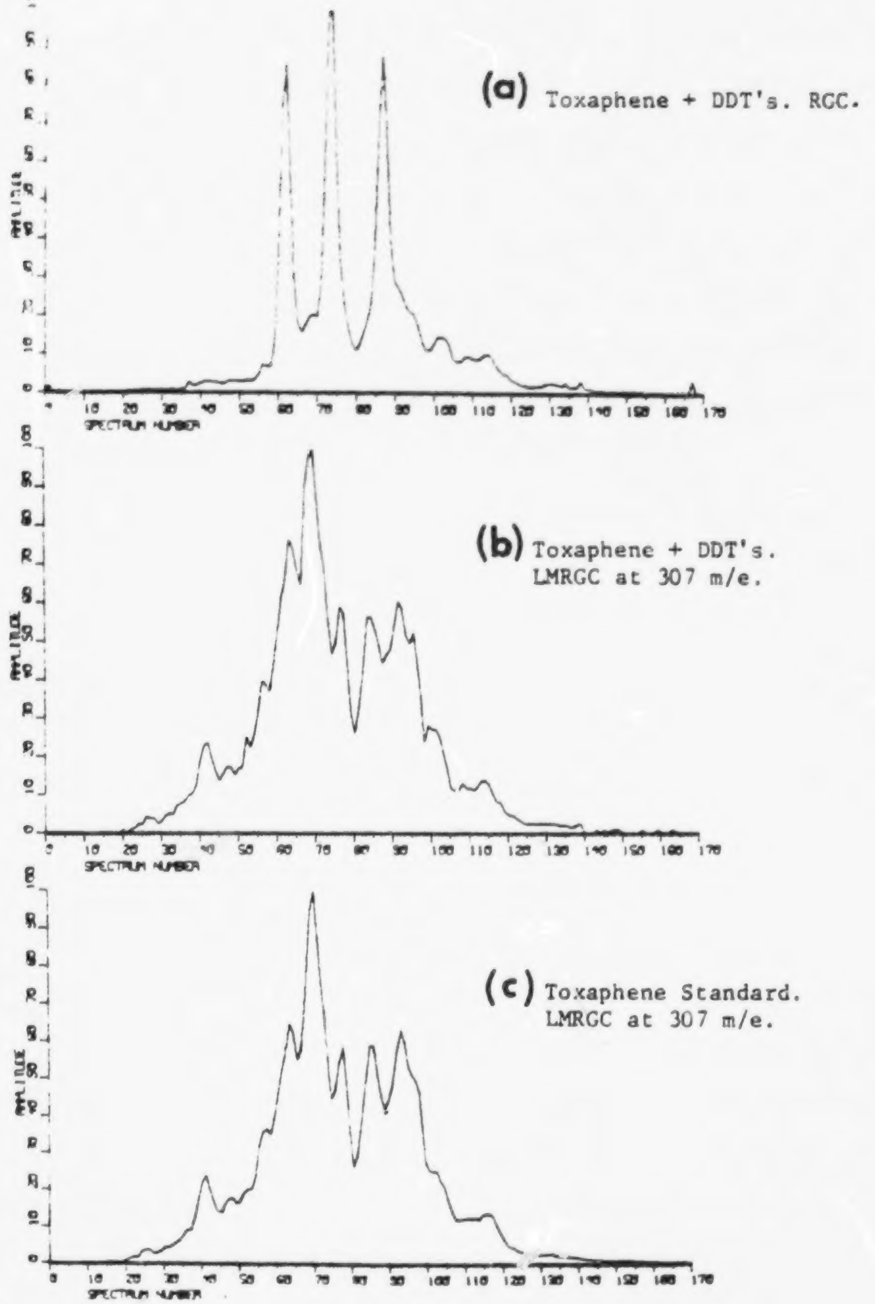
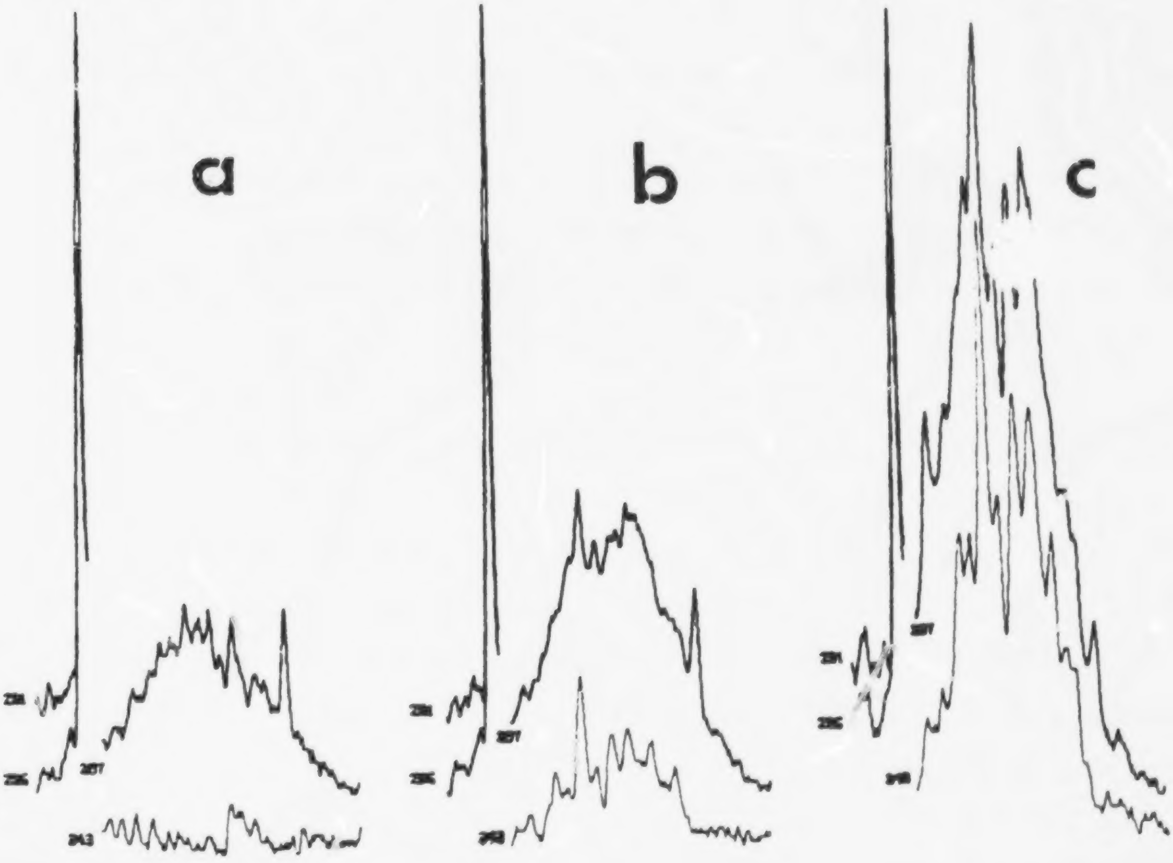


Figure 2.5. Specific ion monitoring with gas chromatography of toxaphene and DDT residues. Source: Thurston, 1976.



a: New Orleans Drinking Water Extract. 2 µl of 1:25,000 $CHCl_3$ solution plus 0.3 ng internal standard TCB.

b: New Orleans Drinking Water Extract. 2 µl of 1:25,000 $CHCl_3$ solution plus 0.3 ng internal standard TCB plus 5 ng toxaphene.

c: New Orleans Drinking Water Extract. 2 µl of 1:25,000 $CHCl_3$ solution plus 0.3 ng internal standard TCB plus 15 ng toxaphene.

Figure 2.6. LMRGC for toxaphene in New Orleans drinking water extract. Source: Thurston, 1976.

Moats (1966) prepared TLC plates at 250 microns thickness from aluminum oxide G or H, or silica gel G or H by adding 0.2 g silver nitrate (dissolved in aqueous alcohol) to a slurry of 30 g adsorbent in 200 to 300 ml water. The samples were cleaned up by Florisil chromatography prior to analysis. Toxaphene chromatographed on aluminum oxide G with hexane development yielded a streak from R_f 0.12 to 0.40.

Ismail and Bonner (1974) have developed a similar RPTLC. They treated 30 g of aluminum oxide with 60 ml of 0.2 percent nitric acid and 10 ml of 1 percent aqueous silver nitrate. Their plates are subsequently coated to no more than 250 microns thickness, air dried overnight, heated to 80 C for 30 minutes, cooled, and then saturated with 8 percent liquid paraffin in petroleum ether. Their mobile solvent is acetonitrile-acetone-methanol-water (40:18:40:2), which is saturated with paraffin oil. Color was developed by exposing the plate to light. Toxaphene (2.5 micrograms) appeared as a characteristic series of spots.

Faucheux (1965) used diphenylamine-zinc chloride for detecting toxaphene and 33 other pesticides. Toxaphene was chromatographed on aluminum oxide and developed with n-heptane. The chromogenic reagent yielded a grayish-green streak for toxaphene (and Strobane) which would be distinguished from chlordane (a purple streak) and other insecticide residues. Lower limit of detection is ca. 3 micrograms.

2.6.4.3 Colorimetry--The product obtained by reacting toxaphene with diphenylamine distinguishes toxaphene from other pesticides and has also been used for quantitative analysis (Graupner and Dunn, 1960; Faucheux, 1965; Adamovic, 1968; Klein and Link, 1970; Thielemann, 1973). Table 2.11 compares the product color from toxaphene with colors produced with other pesticides. Graupner and Dunn (1960) reported that toxaphene has an absorption maximum at 640 nm.

Table 2.11. Comparison of colors obtained by the reaction of pesticides with diphenylamine-zinc chloride[a]

Pesticide	Product color
Toxaphene	Bluish-green
Rothane	Yellow
Lindane	Colorless
DDT	Orange-green
Endrin	Green
Heptachlor	Green
Methoxychlor	Yellow-green
Aldrin	Purple
Dieldrin	Purple
Chlordane	Purple-blue
Kelthane	Purple

[a] Source: Adapted from Graupner and Dunn, 1960, and Faucheux, 1965.

Klein and Link (1970) suggest the following procedure for toxaphene analysis. The sample is cleaned up by the sulfuric-fuming nitric acid method. To ca. 20 micrograms of toxaphene residue are added 0.2 ml each of p-diphenylamine sulfonic acid sodium salt in methanol, and 0.50 percent zinc chloride in acetone. The solvent is stripped and the residue heated for 2 minutes at 210 C. The residue is dissolved with 10 ml ethanol and the absorbance is read at 625 nm. Concentrations measured by this method (down to ca. 0.15 ppm) were approximately 10 percent higher than from GC (electron capture detection).

2.6.4.4 Total Chlorine Determination--Toxaphene concentrations can be determined by measurement of liberated chloride. The method is useful only where other sources of chloride ion will not interfere. The method is not practical for measuring ambient samples, but is discussed because of its historical importance before GC was introduced. Chloride determination can be separated into two parts: chloride liberation and chloride measurement.

Chloride has been quantitatively liberated from toxaphene by combustion (Schoniger flask) and active metal reactions (Guyer et al., 1971; Hartwell et al., 1974). Active metal systems used for this purpose include sodium-isopropyl alcohol, sodium-liquid ammonia, sodium biphenyl, and zinc-acetic acid (Guyer et al., 1971; Hornstein, 1957). Analytical methods for the generated chloride ion include amperometric titration using silver coulometry, spectrophotometric analysis using mercury thiocyanate-ferric ion, and specific ion electrode (Guyer et al., 1971; Widmark, 1971).

2.6.5 Recommended Toxaphene Analysis

Table 2.12 summarizes the recommended method of toxaphene analysis, as adapted from Hartwell et al. (1974) and Guyer et al. (1971). Hartwell et al. (1974) recommended the use of 5 percent SE-30 and 5 percent DC-710 on Gas Chrom Q

Table 2.12. Recommended procedure for toxaphene measurement[a]

Procedure	Function
Extract the sample with n-hexane.	
Chromatographic clean-up by eluting the hexane solution through sulfuric acid/Celite 545 column with 100 ml of n-hexane	Removes natural products (e.g., waxes, fats).
Optional chromatographic clean-up on Florisil (6% ethyl ether/petroleum ether).	Removes PCB and other chlorinated hydrocarbon interferences.
Dehydrohalogenate by evaporating the hexane and treating the residue with 25% KOH in aqueous ethanol at 75 to 80 C for 15 minutes.	DDT and other chlorinated organics do not interfere with GC analysis of the derivative.
Extract by diluting the alkaline mixture with distilled water. Extract with 0.5 ml n-hexane.	
Gas chromatography (electron capture or microcoulometric detection). Column: 9' x 1/8" i.d. glass of 1:1 mixture 5% SE-30 and 5% DC-710 on Gas Chrom Q Operating conditions: 200-210 C	

[a] Source: Adapted from Guyer et al. (1971) and Hartwell et al. (1974)

as the column and recommended it be conditioned for two days at 250 C. The method is designed for standard GC detection systems: microcoulometer or electron capture. If mass spectral specific ion monitoring is used, the dehydrohalogenation step should be eliminated.

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3.0 BIOLOGICAL ASPECTS IN MICROORGANISMS

3.1 SUMMARY

Toxaphene does not significantly affect soil microorganisms - bacteria and fungi, or microbiological processes important to soil fertility - e.g., nitrification, organic matter decomposition, nodulation of legumes - at concentrations even higher than those used for controlling soil insects. At lower concentrations, toxaphene stimulates growth, carbon fixation, or cell population of microorganisms.

Unicellular freshwater- and marine algae are quite suceptible to toxaphene; growth and photosynthesis are inhibited at fairly low concentrations (~ 1 ppm). Marine algae in general are more susceptible than freshwater algae. No single, isolated fraction of toxaphene is exclusively responsible for toxicity. One toxic fraction has been partially characterized as an octachlorobornane. Toxaphene is also inhibitory to freshwater protozoans at a concentration of 60 ppb.

Bacteria, fungi, and unicellular algae are unable to degrade detectable quantities of toxaphene. The microorganisms, however, sorb significant amounts of the pesticide rapidly from the medium. The values of the distribution constant for various microorganisms for toxaphene were in the range of 3.4 to 17.0.

3.2 BACTERIA

Toxaphene, an agricultural insecticide, is applied predominately to soil for the control of soil insects. Its interaction with soil bacteria has received the most attention. Studies dealing with the effect of toxaphene on soil bacteria and soil bacterial processes are summarized in Table 3.1 and Table 3.2.

Table 3.1. Effect of field application of toxaphene on soil bacteria

Reference	Type of soil	Concn. of toxaphene	Period of treatment	Effect on numbers of soil bacteria (some studies include actinomycetes)
Bollen et al., 1954	1. Chehalis silty clay loam 2. Lake labish peat	11.2 to 22.4 kg/ha	10 to 20 days	No measurable effect
Martin et al., 1959	1. Ramona sandy loam 2. Holtville sandy clay loam	22.4 kg/ha, 5 annual applications	5 to 6 years	No measurable effect
Eno et al., 1964	Leon fine sand	16.8 kg/ha, 12 semi-annual applications	3 to 6 years	No measurable effect
Eno and Everett, 1958	Arrendondo loamy fine sand	12.5 to 100 ppm	1 to 16 months incubation in greenhouse	No measurable effect
Smith and Wenzel, 1948	Branchville sandy loam and cadorus silt loam (with and without cottonseed meal)	250 to 1250 ppm (simulating 112 to 560 kg/ha acre in the field)	5 to 96 days incubation in greenhouse	Stimulation of growth
Elfadl and Fahmy, 1958	Silt loam soil	4.9 to 40.3 kg/ha	30 to 136 days incubation in greenhouse	Lower concns: stimulatory; Higher concns.: somewhat inhibitory

Table 3.2. Effect of toxaphene on processes in soil catalyzed by bacteria

Reference	Type of soil	Soil microbial function examined	Concn. of toxaphene	Period of treatment	Effect
Eno and Everett, 1958	Arredondo loamy fine sand	Nitrification, CO_2 evolution, nodulation of legumes	12.5 to 100 ppm	1 to 16 months in greenhouse	Stimulation of nitrification and CO_2 evolution; no significant effect on nodulation
Martin et al., 1959	1. Ramona sandy loam 2. Holtville sandy clay loam	Organic matter decomposition, nitrification	22.4 kg/ha	5 to 6 years	No effect
Eno et al., 1964	Leon fine sand	Nitrification CO_2-production urea hydrolysis	16.8 kg/ha, 12 semi-annual applications	3 to 6 years	No significant effect
Elfadl and Fahmy, 1958	Silt loam	Nodulation of legumes	4.9 to 40.3 kg/ha	30 to 136 days incubation in greenhouse	Stimulation at low doses up to 9 lbs/acre; No effect at higher concentrations

3.2.1 Metabolism

The uptake and metabolism of toxaphene in bacteria has been reported by Paris et al. (1975) in some detail; this is the only study on the subject. The test organisms used included a gram negative bacterium, *Flavobacterium harrisonii*, isolated from citrus plant effluent, and a gram positive bacterium, *Bacillus subtilis*, isolated from Soya Creek. The bacteria were unable to degrade detectable quantities of toxaphene. The sorption of toxaphene by bacteria, as determined by following the decrease in pesticide concentration in the culture medium, was rapid. The cultures reached equilibrium with toxaphene in the medium within 30 minutes and no further change was noted over 24 hours. Extraction of toxaphene from the cells accounted for all the pesticide in the parent form. Sorption of pesticide followed the Freundlich equation; the values of the distribution coefficient (mg pesticide per mg dry wt of the org[illegible] mg pesticide per mg water) were calculated to be 3.4 and 5.2 for *B. subtilis* and *F. harrisonii*, respectively, which shows that the *B. subtilis* sorbed toxaphene less effectively than *F. harrisonii*.

When microorganisms with sorbed pesticides move to aqueous environments, they release some of the compound to the environment. The sorption in *B. subtilis* and *F. harrisonii* was reversible (Paris et al., 1975). Desorption of equilibrium was achieved in the same time with comparable k values.

3.2.2 Effects

Information concerning the interaction of toxaphene with soil bacteria has been derived largely from field studies. Bollen et al. (1954) added 11.2 -22.4 kg of toxaphene per hectare to field soils. After 10 and 20 days, no effect on numbers of bacteria and streptomyces was noted. In another study, five annual applications of toxaphene at 22.4 kg/ha in the field soils produced no measurable effect on numbers of soil bacteria during the five year period and up to 10 months after the fifth application (Martin et al., 1959). Other researchers (e.g., see Eno et al., 1964) have also found no measurable change in soil bacterial population following addition of toxaphene. This inference is also supported from the studies carried out under controlled conditions in a greenhouse (Elfadl and Fahmy, 1958; Eno and Everett, 1958). A few studies have shown that toxaphene stimulates bacterial growth in soil at low concentrations (Smith and Wenzel, 1948; Elfadl and Fahmy, 1958; Eno and Everett, 1958). The stimulatory effect may be due to a catalytic effect of small quantities of toxaphene on certain physiological processes of the cell, or to a favorable shift in the ecological balance in the soil, e.g., destruction of certain predators (Eno and Everett, 1958). The actinomyces, on the other hand, failed to show any increase due to the insecticide.

The effect of toxaphene on the microbiological processes important to soil fertility, e.g., nitrification, organic matter decomposition, etc., has been studied by many researchers. Eno and Everett (1958) reported stimulation of nitrification (∿ 10%) and CO_2-evolution-rate (∿ 8%) one month after application of toxaphene to soil. The concentration of toxaphene ranged from 12.5 to 100 ppm (approximately equivalent to 28-224 kg/ha of the active ingredient of the insecticide). Sixteen months after insecticide application, no significant change occurred in the microbiological processes. No significant effect by toxaphene on soil microbiological processes was demonstrated by other investigators (Eno et al., 1964; Martin et al., 1959).

To evaluate the effect of toxaphene on nodulation of legumes, Eno and Everett (1958) treated soil with toxaphene and 35 days later planted with

Rhizobium-inoculated sweet clover seed. An inspection for nodules 10 weeks later revealed no significant effect on nodulation on the clover grown in treated soil. Similarly, Elfadl and Fahmy (1958) noted no effect of toxaphene at the higher concentrations tested (40.3 kg/ha) on nodulation of Rhizobium-inoculated cowpea. At lower concentrations of toxaphene (< 10 kg/ha), an increase of 20% to 40% was observed in the number of nodules per plant.

3.3 FUNGI

3.3.1 Metabolism

The degradation and accumulation of toxaphene by a pure culture of fungi has been briefly investigated by Paris et al. (1975). In this study, an Aspergillus sp. was isolated from a chicken plant effluent on Rose Bengal agar. Subsequently, it was grown on glucose in the presence of toxaphene. The fungus was unable to degrade detectable amounts of toxaphene during a 12 week incubation period. The sorption of toxaphene by Aspergillus sp. was relatively slow with the cultures reaching equilibrium with the medium in nearly two hours. The fungi formed clumps while growing but the time required for equilibration was independent of the clump size. The sorption of the insecticide followed the Freundlich equation, and the value of distribution coefficient was calculated to be 17.0, which is higher than for bacteria (Section 3.2). This suggests that fungi sorb toxaphene more effectively than bacteria. The ability of toxaphene to desorb from fungi when placed in toxaphene-free environment was not investigated.

3.3.2 Effects

A large number of the reported studies have dealt with the effect of toxaphene on soil fungi. The experimental conditions and results of the studies are summarized in Table 3.3.

Table 3.3. Toxicity of toxaphene to soil fungi

Reference	Type of soil	Concn. of toxaphene	Period & conditions of treatment	Effect on growth
Eno and Everett, 1958	Arredondo loamy fine sand	12.5 to 100 ppm	1 to 16 months incubation in greenhouse	0 to 22% stimulation
Smith and Wenzel, 1948	Brandville sandy loam and cadorus silt loam (with and without cottonseed meal)	250 to 1250 ppm (simulating 112 to 560 kg/ha in the field)	5 to 96 days incubation in greenhouse	Stimulation - cell number more than doubled
Eno et al., 1964	Leoni fine sand	16.8 kg/ha, 12 semi-annual applications	3 to 6 years, field application	No measurable effect
Martin et al., 1959	Ramona sandy loam Holtville sandy loam	22.4 kg/ha, 5 annual applications	5 to 6 years, field application	No measurable effect
Bollen et al., 1954	Chehalis silty clay loam Lake labish peat	11.2 to 22.4 kg/ha	10 to 20 days, field application	Stimulatory at 10 lbs/acre; No significant effect at 20 lbs/acre

As can be seen in Table 3.3, the toxicity of toxaphene to soil fungi has been assessed largely from field and greenhouse studies. Field application of toxaphene of 16.8-22.4 kg/ha has commonly not adversely affected fungi (Eno et al., 1964; Martin et al., 1959). In fact, in a number of studies, toxaphene has increased fungal cell numbers (Smith and Wenzel, 1948; Eno and Everett, 1958, Bollen et al., 1954). Smith and Wenzel (1948) noted nearly doubling of the cell numbers by toxaphene in a greenhouse study at concentrations simulating 112-560 kg/ha toxaphene application in the field. The normal application rate of toxaphene as an insecticide is only 1.12-6.7 kg/ha.

A differential effect of toxaphene on three species of fungi was noted by Bollen et al. (1954). Field application of toxaphene at 11.2 kg/ha to a peat soil caused an overall increase of 62% in the total number of fungi. A study of the comparative effect of toxaphene on three genera of fungi (Penicillium, Aspergillus, and Mucor) revealed that toxaphene caused an increase in the counts of Penicillium, whereas the numbers of Aspergillus and Mucor decreased. Similar changes were caused by toxaphene at 20.4 kg/ha; however, the effect was less pronounced. Conversely, Martin et al. (1959) have noted no significant influence of toxaphene on numbers or kind of soil fungi in sandy loam or sandy clay loam soil. The findings of Bollen et al. (1954) may be suggestive of the potential of toxaphene for altering microbial number and affecting microbial community structure in certain soils.

3.4 UNICELLULAR ALGAE

Unicellular algae constitute an important constituent of the food chain and a broad base for primary productivity in our aquatic environment. Consequently, it is important to evaluate the effect of environmental contaminants on freshwater and marine algae.

The interaction of toxaphene with freshwater and marine algae has been the subject of numerous studies. The effect on growth and photosynthetic processes of the cell have been studied most frequently.

3.4.1 Uptake and Metabolism

Information concerning uptake and metabolism of toxaphene in unicellular algae is virtually nonexistent. Paris et al. (1975) briefly examined the extent of sorption of toxaphene by the green alga, *Chlorella pyrenoidosa*. The culture of algae was analyzed at intervals by centrifuging an aliquot and determining the pesticide concentrations in the supernatant. The algae was noted to be in equilibrium with the medium within 10 minutes. The sorption of toxaphene was represented by the empirical equation of Freundlich; the value of the distribution constant (mg pesticide per mg dry wt of the organism/mg pesticide per mg water) for toxaphene by algae was 17.0. Toxaphene sorption was also demonstrated in field samples containing algae (*Scenedesmus* sp. and *Chlorella* sp.) and other microorganisms. The extraction and analysis of the whole algal cultures revealed no degradation of toxaphene by green algae after 24 hours of incubation.

In an experiment designed to determine bioaccumulation/biomagnification potential of toxaphene, Schoettger and Olive (1961) exposed cultures of the planktonic alga, *Scenedesmus incrasstulus*, to 0.01 ppm toxaphene for 384 hours. The accumulated toxaphene was extracted from algae with benzene, the extract redissolved in isopropyl alcohol. The presence of toxaphene in the extract was bioassayed using *Notropis* sp. of fish as the test organism. The results indicated that the algae had not accumulated a sufficient quantity of toxaphene to kill any of the test fish (Table 3.4). On the other hand, periphytons (composed

Table 3.4. Accumulation of toxaphene in fish-food organisms exposed to multiple toxaphene dosages[a]

Daily dosage of toxaphene (ppm)	Toxaphene formulation used	Exposure Period (days)	No of cells/liter	% Fish surviving up to 120 hrs. (Test fish: Notropis sp.)
Planktonic algae - as fish-food organism				
0.01	100% technical grade	16	$1,850 \times 10^5$	100
0.01	40% wettable powder	16	$3,300 \times 10^5$	100
Periphyton (composed of Cladophora sp., Anacystis sp., Scenedesmus sp., Novicula sp., Diatoma sp., and Ciliophora sp.) as fish-food organisms				
0.01	100% technical grade	13	Not specified	0
0.01	40% wettable powder	13	Not specified	0

[a] Source: Modified from Schoettger and Olive, 1961.

of various algae, diatoms, and ciliates) exposed to toxaphene, extracted and bioassayed in a similar manner, revealed concentrations of toxaphene high enough to kill fish within 24 hours. It was suggested that periphyton accumulated toxaphene by incorporating it into the mass rather than by adsorption.

3.4.2 Effect on Growth

The salient features of the studies dealing with the effect of toxaphene on growth are given in Table 3.5. Ukeles (1962) tested the toxicity of toxaphene on five species of marine algae. A toxaphene solution prepared in sterile sea water was added in appropriate concentration (final concn. 0.01-0.15 ppm) to the sterile basal medium which had been supplemented with the test species of algae. The algal cultures were incubated under continuous illumination and growth rates were measured by the determination of transmittance at 530 nm. The concentrations of toxaphene required to cause complete inhibition of growth of the five species of marine phytoplankton are shown in Table 3.5. Of the various species, the Monochrysis sp. was the most sensitive to toxaphene since complete inhibition of growth was observed at a toxaphene concentration as low as 10 ppb. Other species were also inhibited by toxaphene but at relatively higher concentrations.

Unlike the effect of toxaphene on marine algae, the effect on freshwater algae has been studied by a number of researchers. The earliest study was that of Palmer and Maloney (1955) who studied the effect of toxaphene at a concentration of 1.5 ppm on several laboratory strains of unicellular algae representing blue-greens, greens, and diatoms. The criteria for selecting the algal species was their ability to produce rapid and uniform growth under laboratory conditions. The effect of toxaphene was assessed in a qualitative

Table 3.5. Toxicity of toxaphene to unicellular algae

Reference	Test *sp*.	Concn. of toxaphene (ppm)	Period of treatment (days)	Effect on growth
A. *Marine algae*				
Ukeles, 1962	*Proloccus* sp.	0.15	10	100% inhibition
	Chlorella sp.	0.07	10	100% inhibition
	Dunaliella sp.	0.15	10	100% inhibition
	Phacodactylum tricornutum	0.04	10	100% inhibition
	Monochrysis lutheri	0.01	10	100% inhibition
B. *Freshwater algae*				
Palmer and Maloney, 1955	1. Blue-green algae	1.2	up to 21 days	
	Cylindrospermum licheniforme			Partially toxic
	Microcystis aeroginosa			Toxic
	2. Green algae	"	"	
	Scenedesmus obliquus			Partially toxic up to 7 days, non-toxic afterwards
	Chlorella variegata			Non-toxic
	3. Diatoms	"	"	
	Gomphonema parvulum			Partially toxic
	Nitzschia palea			Partially toxic up to 7 days, non-toxic afterwards

Table 3.5 (continued)

Reference	Test sp.	Concn. of toxaphene (ppm)	Period of treatment (days)	Effect on growth
B. Freshwater algae (continued)				
Stadnyk et al., 1971	Green algae Scenedesmus quadricaudata	0.1	Up to 10	No significant decrease in cell number and biomass
	"	1.0	Up to 10	19% decrease in cell number, no significant effect on biomass
O'Kelley and Deason, 1976	21 strains[a] from the Warrier River	0.001	14	12% strains inhibited (growth >50%) 6% no effect 3% stimulated (growth >110%)
		0.01	14	2% strains inhibited (growth >50%) 13% no effect 6% stimulated (growth >110%)
		0.1	14	1% strains inhibited (growth <50%) 7% inhibited (growth >50%) 8% no effect 5% stimulated (growth >110%)

Table 3.5. (continued)

Reference	Test sp.	Concn. of toxaphene (ppm)	Period of treatment (days)	Effect on growth
B. Freshwater algae (continued)				
O'Kelley and Deason, 1976 (continued)	21 strains[a] from the Warrier River (continued)	1.0	14	8% strains inhibited (growth <50%) 7% inhibited (growth >50%) 6% no effect

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[a] Belonging to Chlorella sp.; Nitzschia sp.; Scenedesmus sp.; Carteria sp.; Koliella sp., Actinastrum sp., Monoraphidium sp., and Golinkinlopsis sp.

manner by comparing the amount of visible growth. The authors noted no effect on Chlorella (a green algae); however, the growth of other algal cultures was partially to fully inhibited at 1.5 ppm toxaphene.

The fresh water algae as a group are very heterogeneous, both physiologically and morphologically. Predictions of pesticide interaction with algae in the field, based upon the data with common laboratory strains, may be subject to error. Several researchers have realized this difficulty and have used strains of algae isolated from streams, lakes, and ponds in their toxicity studies. Stadnyk et al. (1971) used a field-collected strain of green alga, Scenedesmus quadricaudata, for studying toxaphene toxicity. The test medium was supplemented with micronutrients to approximate the conditions of a eutrophic lake. The authors reported no significant effect of toxaphene at concentrations ranging from 0.1 to 1 ppm on cell number or biomass of the plankton algae. However, carbon fixation in Scenedesmus was markedly stimulated in the presence of toxaphene (Section 3.3.2). In a more elaborate study, O'Kelley and Deason (1976) tested the influence of toxaphene on the growth of 21 strains of algae which were isolated from the Warrior River near Tuscaloosa, Alabama. Following treatment, cultures were incubated in a growth chamber without aeration. Growth was measured turbidimetrically at the end of two weeks. Only a few algal isolates were inhibited by 0.001 or 0.01 ppm of toxaphene; however, significant numbers were inhibited when the concentration was increased to 0.1 to 1.0 ppm. At toxaphene concentrations up to 0.1 ppm, a small proportion of the isolates (∿ 5%) showed stimulated growth (Table 3.5).

Matsumura and coworkers (Nelson and Matsumura, 1975a) have attempted to isolate and identify the components of toxaphene which are toxic to the blue-green alga, Anacystis nidulans, and other aquatic organisms. The components were separated by a Sephadex LH-20-methanol column and thin-layer chromatography. Following the addition of the separated fractions, the algal cultures were incubated in light and aerated with air enriched to 2% CO_2 (v/v). The growth rates were calculated from short term experiments lasting 26 to 30 hours. The relative toxicity values were obtained by comparing growth rates in standard toxaphene with separated fractions at a concentration of 1 ppm. Toxaphene at this concentration caused nearly 1/3 reduction in the growth rates of the algae. Figure 3.1 shows that no single, isolated fraction was exclusively responsible for toxaphene toxicity, and that the activity was quite evenly distributed among several fractions. This led the authors to conclude that several components of toxaphene are toxic to algae, and that the toxic action is relatively non-specific. A toxic fraction which was nearly 1.35 times more toxic than toxaphene was partially characterized as an octachlorobornane.

Nelson and Matsumura (1975b) used a simplified approach to study toxic fractions of toxaphene. They chlorinated purified exo-2,10-dichlorobornane, a major early chlorination product of camphene, to form a "simplified toxaphene" which was then used for isolation of toxic components. The four chlorination products purified were only slightly toxic to the blue-green alga, Anacystis nidulans, at 1 ppm (growth constant for chlorination products = 0.76 to 0.93 per day, compared to control, 0.96 per day). This was in contrast to toxaphene, which strongly inhibited algal growth at similar concentrations (growth constant = 0.32 per day), and suggested that exo-2,10-dichlorobornane was not a precursor for the components of toxaphene which are toxic to algae.

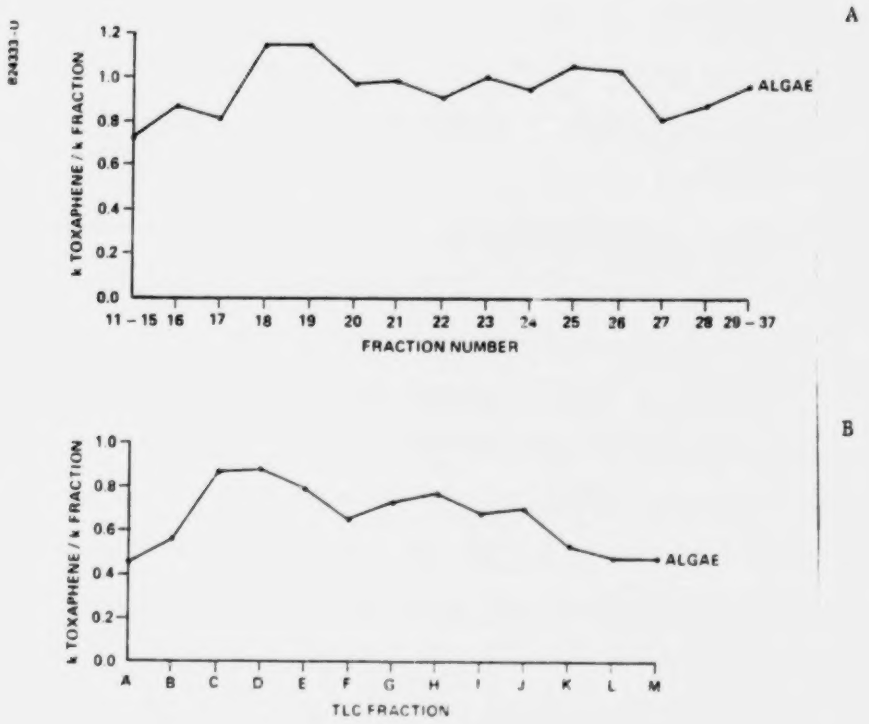

Figure 3.1. Relative toxicity of toxaphene components as compared with toxaphene (as 1) to freshwater blue-green algae, Anacystis nidulans. Source: Nelson and Matsumura, 1975 a.

k values represent growth rate; k value ratios were calculated at toxicant concentrations of 1 ppm. Average k value for toxaphene = 0.327 ± 0.009; k value for control = 0.96 ± 0.072.

A. Fractions collected from Sephadex LH-20 - methanol column.

B. Fractions separated by thin layer chromatography.

3.4.3 Effect on Physiological Processes

Information is available concerning the effect of toxaphene on the photosynthetic process of the cell (Table 3.6). This physiological process has received attention because of the fact that an alteration of the photosynthetic process at the algal level is ultimately reflected in altered production at higher trophic levels.

The effect of toxaphene on the photosynthetic process has generally been assessed by examining the effect on C-14 labelled CO_2-assimilation in the cell. In a four hour exposure of toxaphene at 1 ppm, Butler (1963) reported almost complete inhibition of carbon fixation in estuarine phytoplankton (no details of species given) (Table 3.6). Stadnyk et al. (1971) measured the effect of toxaphene on C-14 labelled CO_2-assimilation of the plankton algae, Scenedesmus quadricaudata, obtained from field collection. The experimental procedure involved withdrawal of aliquots of toxaphene-treated culture at different intervals; the incubation of cultures with C-14 labelled Na_2CO_3 for four hours; and the determination of C-14 in the cells. Toxaphene was found to cause a marked stimulation of carbon fixation. As much as 450% increase in carbon fixation was observed at 1 ppm toxaphene. The degree of stimulation decreased with continued incubation with toxaphene. The mechanisms of carbon-fixation stimulation by toxaphene is unclear at the present time.

3.5 EFFECT OF TOXAPHENE ON PROTOZOA

A field study concerning the effect of toxaphene on protozoa and other plankton in a reservoir has been described by Hoffman and Olive (1961). The reservoir, a single basin lake with a surface area of 24.3 ha and a depth of 15 feet, was treated with toxaphene at a concentration of 0.06 ppm and the

Table 3.6. Effect of toxaphene on the physiological processes of unicellular algae and phytoplankton

Reference	Test species	Concn. of toxaphene tested (ppm)	Period of treatment	Physiological process studied	Effect observed
Butler, 1963	Estuarine phytoplankton (no other details given)	1	-	CO_2-fixation	91% inhibition
Stadnyk et al., 1971	Green algae *Scenedesmus quadricaudata*	0.1	Up to 10 days	CO_2-fixation expressed as per unit culture volume, or per cell	Initially 48% increase, no significant effect afterwards
"	"	1.0	"	"	Initially 450% increase, 30% increase after ten days

number of plankton organisms was determined for a year period, starting in October. The authors noted that, following the application of toxaphene to the reservoir, the turbidity decreased to the point that the bottom became visible. This suggested a toxic action of toxaphene on the aquatic microorganisms. Protozoans decreased from high counts taken in October to nearly zero in December. Furthermore, in the control reservoir the number of protozoan increased, whereas the number in the treated reservoirs remained at nearly zero. These results suggest that toxaphene was inhibitory to protozoans and other microorganisms at the concentration studied.

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4.0 BIOLOGICAL ASPECTS IN PLANTS

4.1 SUMMARY

The information concerning the interaction of toxaphene with plants has been confined to vascular crop and noncrop plants. Aquatic plants are able to accumulate appreciable amounts of toxaphene from the medium. That toxaphene can accumulate in plants is also demonstrated by the fact that toxaphene in large quantities has been detected in the aquatic weeds collected from the vicinity of a toxaphene discharge point. Considerable toxaphene residues have also been detected in various crop plants - beet leaves and roots, cabbage, collard, ladino clover seed greenings, onion greens, and snap beans - when these crops have received a toxaphene application. The residues were higher in most crops immediately after harvest, but decreased greatly upon incubation. A significant reduction in toxaphene residues occurs during commercial packing house washing and in food processing. In general, detergent washing removed more toxaphene than washing with water. A survey by FDA shows that human exposure to toxaphene via food is not appreciable.

In spite of the fact that toxaphene is not recommended for use in tobacco, significant quantities have been detected in tobacco and tobacco products. Toxaphene caused off-flavor in tobacco when applied to tobacco foliage; however, soil applications of toxaphene produced no significant effect on flavor.

Toxaphene is not phytotoxic to most crop plants at concentrations recommended for control of insects (15 to 20 kg/ha, annual). The growth and development of cauliflower, cabbage, and tomato has, however, been shown to be affected to some extent at such concentrations of toxaphene. At concentrations 10 to 20 times the normally recommended dose, detrimental effects of toxaphene on oat plants have been noted. No information concerning the effect of toxaphene on noncrop plants is available.

Toxaphene generally does not impart off-flavor to raw and canned vegetables. Some adverse effect was noted in cooked rutabagas, onions, and carrots. Application of toxaphene to the soil appears to cause more instances of changes in flavor than did applications to the foliage. It is believed that crop residues result primarily from surface deposition, and only little if any translocation of toxaphene occurs in plants.

4.2 NONVASCULAR PLANTS - HIGHER FUNGI, MACRO-ALGAE, AND MOSSES

No studies were encountered in the literature which dealt with the interaction of toxaphene with nonvascular plants. Among the miscellaneous samples analyzed for toxaphene by Reimold and Durant (1972 a) was the macro-algae Ulva (sea lettuce). The samples were collected from a Georgia estuary into which toxaphene wastes (from a manufacturing plant) were discharged. Significant concentrations of toxaphene were not detected in the algae in this study (detection limit 0.15 ppm).

4.3 VASCULAR PLANTS

4.3.1 Noncrop Plants

The effect and metabolism of toxaphene in noncrop plants has not been investigated extensively. In view of the fact that toxaphene has been suggested for use in rehabilitation of fresh water fishing lakes and reservoirs, a number of the reported studies have dealt with the distribution and analysis of toxaphene in aquatic fauna and flora following application of toxaphene for fish eradication. Some information is also available on the levels of toxaphene in vegetation in toxaphene-contaminated areas. Virtually no information on the effect of toxaphene on noncrop plants is available.

4.3.1.1 Metabolism: Uptake, Adsorption, and Residue -- Information on the metabolism and fate of toxaphene in noncrop plants is important in the overall assessment of the environmental hazards of toxaphene. Noncrop plants may hold toxaphene and/or toxaphene metabolites, and recycle them into water by elution or lysis. Also, the terrestrial and aquatic animals feeding on the vegetation may ingest toxaphene and concentrate it in their bodies. Reimold (1974), while studying the levels of toxaphene in organisms collected from the contaminated estuary, noted that the salt marsh cordgrass, _Spartina alterniflora_, contained 36.3 ppm toxaphene in leaves, 4.9 ppm in the seed heads, and 1.9 ppm in the roots. The salt marsh sediment in the same area contained only 32.5 ppm toxaphene. Realizing that the high toxaphene content of _S. alterniflora_ represents a translocation of toxaphene from sediment into the plant tissue, the author undertook a detailed study of the movement of toxaphene through the salt marsh sediment into _S. alterniflora_ using Cl-36-labelled toxaphene. The plants collected from a marsh were placed in sea water medium containing toxaphene and, following various incubation periods, Cl-36 activity and toxaphene concentration in various parts of the plant were determined. The toxaphene content in the plant at 26 days represented nearly 20 to 25 percent of the total insecticide initially present in the sea water culture medium (Table 4.1). The insecticide content decreased on continued incubation. The data further showed that the plant roots and rhizomes sorbed greater amounts of toxaphene than leaves and stems. Toxaphene and Cl-36 in the plant after a 100-day incubation were 1.8 and 12.6 percent, respectively. The presence of Cl-36 in excess of that due to the presence of toxaphene as detected by the gas chromatographic method suggests that metabolic alteration of the insecticide had occurred.

Residues of toxaphene in aquatic vegetation following application of toxaphene for the purpose of eradication of rough fish have been examined by a number of researchers (Table 4.2). Kallman et al. (1962) reported accumulation of high concentrations of toxaphene in the rooted aquatic weed, _Potamogeton_.

Table 4.1. Uptake of Cl-36-labelled toxaphene by salt marsh cordgrass, *Spartina alterniflora*[a]

Days of incubation	Plant part	% Uptake of available toxaphene[b]	% Uptake of available Cl-36[b]
26	Leaves and stems	0.621 ± .60[c]	2.17 ± 1.4[c]
26	Roots and rhizomes	20.1 + 5.2	22.5 ± 1.6
100	Leaves and stems	0.048 ± .04	0.6 ± 0.36
100	Roots and rhizomes	1.82 ± 0.7	12.02 ± 3.6

[a] Modified from Reimold, 1974.

[b] The sea water culture medium initially contained 100 ppm toxaphene + 4.316 μCi Cl-36 equivalent to 83 mg toxaphene.

[c] Mean ± standard deviation.

Table 4.2 Toxaphene residues in aquatic vegetation in lakes treated with toxaphene for eradication of rough fish[a]

Aquatic vegetation analyzed	Estimated application rate of toxaphene (ppb)	Lake treated	Interval between treatment and analysis	Residue (ppm)	Reference
Potomogeton sp.	10	Clayton Lake, New Mexico	48 hours	2.8	Kallman et al., 1962
	30 (in two applications of 10 and 20)	Clayton Lake, New Mexico	96 hours	4.9	Kallman et al., 1962
	50 (in three applications of 10, 20, and 20)	Clayton Lake, New Mexico	9 days	14.6	Kallman et al., 1962
Aquatic plants (species name not given)	40	Davis Lake, Oregon	4 years	0.39 (0.2-0.6)[a]	Terriere et al., 1966
			5 years	0.21 (0.1-0.9)[a]	
	88 (in two applications)	Miller Lake, Oregon	1 year	4.59 (0.3-15.5)[a]	Terriere et al., 1966
			2 years	2.78 (0.19-7.5)[a]	
			3 years	5.77 (1.87-13.1)[a]	

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[a] Range of detected toxaphene residues.

At the application rate of 50 ppb, the concentration of toxaphene in the aquatic plant was as high as 4.4 ppm after 5 days of incubation, and increased to 14.6 ppm after 9 days. The uptake and fate of toxaphene beyond 9 days was not investigated by the authors. In agreement with these results, Terriere et al. (1966) have also noted accumulation of appreciable residues of toxaphene in aquatic plants. Plants absorbed up to 15.5 ppm toxaphene when growing in water containing approximately 2 ppb of the insecticide (residue detected in water). The analysis of the various parts of the plant revealed that roots usually contained the highest concentration of toxaphene.

The ambient toxaphene levels in aquatic vegetation growing in toxaphene-contaminated areas have been determined by Reimold and Durant (1972 b). The salt marsh cordgrass, Spartino alterniflora, growing in the vicinity of a toxaphene discharge point from a toxaphene manufacturing plant, accumulated toxaphene in the leaves to the extent of 36 ppm (Table 4.3). Other parts of the plant contained relatively low concentrations of toxaphene. Contrary to these findings, the uptake studies of Reimold (1974) with Cl-36-labelled toxaphene had revealed that the plant roots and rhizomes sorbed the greatest amount of toxaphene (Table 4.1). The difference between the two observations was believed to be due to differences in uptake from sea water medium as opposed to marsh soil. The results, however, clearly demonstrate that toxaphene can be concentrated in Spartina when it occurs in the growth medium. Reimold and Durant (1974) also monitored the toxaphene content in salt marsh cordgrass before, during, and after dredging of toxaphene-contaminated sediment and found the concentration to be highest during dredging rather than before or after.

Table 4.3. Ambient toxaphene levels in noncrop plants

Plants analyzed	Sampling location	Source of toxaphene in the vicinity, if any	Toxaphene concentration in the vicinity of the sampling location	Toxaphene levels in plants	Reference
Salt marsh cordgrass	Salt marsh bordering Terry Creek, Brunswick, Georgia (near toxaphene discharge point)	Toxaphene manufacturing plant - Brunswick Operations, Hercules, Inc.	Marsh sediment contained 32.5 ppm toxaphene	16.3 ppm in leaves; 4.9 ppm in seed heads; 1.9 ppm in roots	Reimold and Durant, 1972 b
Salt marsh cordgrass	Locations other than Terry Creek within the Brunswick study area	Toxaphene manufacturing plant - Brunswick Operations, Hercules, Inc.	Marsh sediment contained toxaphene ranging from 0.81 to 63.7 ppm	<0.25–17.14 in leaves; 3.01–6.85 in seed heads; 1.91 in roots; 1.24 in rhizomes	Reimold and Durant, 1972 b
Salt marsh cordgrass	Terry Creek, Brunswick Georgia, before, during, and after dredging operation	Toxaphene manufacturing plant - Brunswick operations, Hercules, Inc.	<10 ppm in surface sediment	Before dredging: 0.9 ppm, during dredging: 0.6–6.8 ppm; after dredging: 2.31 ppm	Reimold and Durant, 1974

4.3.1.2 Effects -- No information was found in the literature concerning the effect of toxaphene on noncrop plants.

4.3.2 Crop Plants

Toxaphene, like other pesticides, can contaminate and accumulate in man's food supplies. Consequently, its interaction with crop plants has received a considerable amount of emphasis. The toxaphene residues remaining in man's food and the adverse effect of toxaphene on plants have been extensively investigated. The information available on these and other aspects of toxaphene interaction with crop plants is summarized below.

4.3.2.1 Metabolism: Uptake, Adsorption, and Residues -- Whereas the uptake and adsorption of toxaphene in crop plants has not been studied, the residues remaining in various crops after applying the insecticide have received a considerable amount of attention. The residues have been determined following both soil and foliar applications.

The conditions of the test and toxaphene residues as found on various crops are shown in Table 4.4. Considerable toxaphene residues were found on beet leaves and roots, cabbage, collards, ladino clover seed greenings, onion greens, and snap benas. Other crops investigated contained only traces or nondetectable levels of toxaphene. In general, lesser amounts of toxaphene were found when the insecticide was applied in soil prior to planting. The residues were higher in most crops immediately after harvest, but decreased greatly upon incubation.

The removal of toxaphene residues from vegetables by washing has been studied by Van Middelem (1966). Detergent washing was found to remove considerably more toxaphene residues from the vegetables tested - tomatoes, green beans, celery, and mustard greens - than the water wash alone (Table 4.5).

Table 4.4 Residue of toxaphene detected in crop plants

Crop studied	Application rate (kg/ha)	Application procedure and number of applications	Interval between treatment and harvest	Residue (ppm)	Reference
Beets	1.7 - 3.3	1-3 dust applications	7-9 days	4.3-41 in tops 3.3-16 in roots	Wene, 1958
Table beet (early wonder)	3.3	Single preplanting treatment	9 days	Below detectable level	Muns et al., 1960
Sugar beet (U.S. 56)	3.3	Single preplanting treatment	18 days	Below detectable level	Muns et al., 1960
Bell peppers	2.8 - 5.6	1-3 dust applications	1 hr-4 wks	0.2-2.9	Wene, 1958
Cabbage	1.1 - 4.4	1-3 applications of dust or spray	1-3 wks	0-5.6	Wene, 1958
Collards	33.6 (20% toxaphene dust)	Single dust application at the time of harvest	0-13 days	4.9-168.0	Brett and Bowery, 1958
Corn	125 - 250	Single application of technical toxaphene	Crop planted 16 yrs after application of insecticide; crop harvested and analyzed at maturity	Nondetectable	Nash and Harris, 1973
Cotton seed Cotton seed oil Cotton meal	123 (wettable powder formulation)	96.9 lbs in a single preplanting application, plus 13.9 lbs in a single foliar application	---	0.1 0.4 0.13	Randolph et al., 1960
Ladino clover seed screenings	1.1 - 1.7 DDT plus 2.2 - 3.3 toxaphene	Two applications	---	21.1 in composite sample	Archer, 1970

Table 4.4 (continued)

Crop studied	Application rate (kg/ha)	Application procedure and number of applications	Interval between treatment and harvest	Residue (ppm)	Reference
Lettuce	2.5 - 3.7	1-3 dust applications	3 hrs-3 wks	0-1.7	Wene, 1958
Oats	125 - 250	Single application of technical grade toxaphene	Crop planted 16 yrs after application of the insecticide; crop harvested and analyzed at maturity	Nondetectable	Nash and Harris, 1973
Onion	1.7 - 3.3	1-3 spray applications	1-3 wks	5-71 in tops; 0.1-4.6 in bulbs	Wene, 1958
Potato (white rose)	3.3	Single preplanting treatment	14 days	0.3	Muns et al., 1960
Snap beans	33.6 (20% toxaphene dust)	Single dust application at the time of harvest	0-12 days	1.5-8.10[a]	Brett and Bowery, 1958
Sorghum	140 (wettable powder)	108 kg/ha in a single preplanting application, plus 31.6 kg/ha in two foliar applications	---	Nondetectable	Randolf et al., 1960
Soybean	125 - 250	Single application of technical grade toxaphene	Crop planted 16 yrs after application of the insecticide; crop harvested and analyzed at maturity	Nondetectable	Nash and Harris, 1973

Table 4.4 (continued)

Crop studied	Application rate (kg/ha)	Application procedure and number of applications	Interval between treatment and harvest	Residue (ppm)	Reference
Stringbeans	2.8 - 5.6	Single dust application	3 hrs-3 wks	0-11.7	Wene, 1958
Tomatoes	33.6 (20% toxaphene dust)	Single dust application at the time of harvest	0-12 days	0.15-4.0[a]	Brett and Bowery, 1958
Wheat	125 - 250	Single application of technical grade toxaphene	Crop planted 16 yrs after application of insecticide; crop harvested and analyzed at maturity	Nondetectable	Nash and Harris, 1973

[a] For comparison, FDA tolerances of toxaphene on snap beans, celery, and tomatoes are 7 ppm (Brett and Bowery, 1968; Van Middelem, 1966).

Table 4.5. Comparison of washing with and without surfactants on removal of toxaphene from vegetables[a]

Washing treatments	Toxaphene, ppm			
	Tomatoes	Green beans	Celery	Mustard green
None	3.69	31.07	60.44	233.64
Water	3.31	24.91	25.81	100.02
0.1% Polyether alcohol	3.19	13.18	76.99	23.09
1% Neutral soap	3.27	12.25	16.42	20.77

[a] Modified from Van Middelem, 1966.

For example, the toxaphene residue on mustard greens which were harvested 12 hours after treatment was reduced 57 percent by water washing alone and 90 percent following the detergent washing. Significant reductions were also noted in toxaphene residues in celery following commercial packinghouse washing (Van Middelem and Wilson, 1960). In food processing, 20 to 60 percent of the residues are removed by processing, washing, peeling, and heating of fruits and vegetables (Hartwell et al., 1974).

Estimates of toxaphene exposure from dietary intake can be made from the U.S. Food and Drug Administration (FDA) market basket survey, the FDA survey of unprocessed food and feed samples, and the U.S. Department of Agriculture (USDA) survey of meat and poultry. In the FDA market basket survey, food samples are prepared for consumption (i.e., cooked, or otherwise processed) prior to monitoring for pesticide residues (Duggan and McFarland, 1967). The market basket items are grouped by commodity class (e.g., dairy products, leafy vegetables, legume vegetables) and are intended to represent a 2-week diet for a 16- to 19-year-old male (Duggan and Corneliussen, 1972). The results of these surveys, from their inception to the most recently published report, are summarized in Table 4.6. From 1964 to 1972, food samples were obtained from five cities: Boston, Massachusetts; Baltimore, Maryland; Los Angeles, California; Kansas City, Missouri; and Minneapolis, Minnesota. Of the 26 positive samples encountered during this period, 19 were in Los Angeles, four were in Baltimore, and one was in Boston. Based on the estimates of daily intake made by Duggan and Corneliussen (1972), and assuming an average body weight of 70 kg, the estimated daily dose of dietary toxaphene over the period of June 1964 to April 1970 was 0.021 μg toxaphene/kg body weight/day. This estimate is based on food samples from a limited number of cities, most of which

Table 4.6. Toxaphene residues found in food and drug administration market basket survey, 1964 to 1975

Monitoring Period	No. of Composite	No. of Composite Positive	% Occurrence	Commodities contaminated (No. of composite of Each commodity contaminated)	Range of levels (mg/kg)	Daily Intake	Reference
June 1964–April 1965	216	0	0.0	—		0	Duggan et al., 1966
June 1965–April 1966	312	3	1.0	Leafy vegetables (1) and garden fruits (2)	0.048–0.38	0.002	Duggan et al., 1967
June 1966–April 1967	360	0	0.0	—	—	0	Martin and Duggan, 1968
June 1967–April 1968	360	4	1.1	Meat, fish, or poultry (1) leafy vegetables (1), garden fruits (2)	0.064–0.375	0.002	Corneliussen, 1969
June 1968–April 1969	360	13	3.6	Garden fruits (6), meat, fish, or poultry (1), legume vegetables (2), root vegetables (1), leafy vegetables (3)	0.022–0.33	0.004	Corneliussen, 1970
June 1969–April 1970	360	4	1.1	Leafy vegetables (2), garden fruits (2)	0.080–0.132	0.001	Corneliussen, 1972
June 1970–April 1971	360	1	0.3	Root vegetables (1)	trace		Manske and Corneliussen, 1974
June 1971–July 1972	420	1	0.2	Leafy vegetables (1)	0.1		Manske and Johnson, 1975
Aug. 1972–July 1973	360	0(1)[a]	0.0	—	(0.005)[a]		Johnson and Manske, 1975
Aug. 1973–July 1974	360	3	0.8	Garden fruits (3)	trace–0.163		Manske and Johnson, 1976
Aug. 1974–July 1975	240	1	0.4	Leafy vegetables (1)	0.118		Johnson and Manske, 1977

[a] Strobane.

are not located in areas of high toxaphene usage. The more recent (1972 to 1975) results of the market basket survey suggest that the current daily dietary dose may be substantially lower; however, it is equally possible that the dietary doses for individuals located in the Mississippi Delta (an area of high toxaphene usage) could be substantially higher. Most toxaphene exposure is associated with leafy vegetables and garden fruits.

Toxaphene residues in significant quantities have also been demonstrated in tobacco and tobacco products (Table 4.7). This is in spite of the fact that toxaphene is not registered for use on tobacco, and its use has been discouraged for many years. Toxaphene is used on cotton, and Domanski and coworkers (1975) state that the residues found in tobacco may be due, at least in part, to drift during application of the insecticide to cotton planted in close proximity to tobacco fields. Over 90 percent of the fire-cured tobacco samples were positive for toxaphene in 1972 (Domanski et al., 1975). This shows a considerable increase from 1970 when only 30 percent of the samples contained toxaphene (Domanski et al., 1973). Significant amounts of toxaphene were found in other types of tobacco also. For example, 50 percent of the 1972 burley samples had toxaphene concentrations greater than 0.5 ppm (Domanski et al., 1975). A survey for toxaphene residues on commercial tobacco products revealed that the insecticide was present in all the products analyzed (Table 4.7). Toxaphene residues in most tobacco products were considerably higher in 1971 than the concentration found in the 1973 auction market samples. For example, toxaphene in cigarettes decreased from 3.3 ppm in 1971 to 1.4 ppm in 1973 (Domanski et al.,

Table 4.7. Toxaphene residues in tobacco and tobacco products

Product studied	Sampling location	Sample details	Residue (ppm)	Reference
Flue cured tobacco	Auction market (1972 crop)	Composite of 50 leaves (5 leaves from each farmer)	0.51–1.96	Domanski et al., 1975; Gibson et al., 1974
Burley tobacco	Auction market (1972 crop)	Composite of 50 leaves (5 leaves from each farmer)	0.43–1.24	Domanski et al., 1975; Gibson et al., 1974
Fire cured tobacco	Auction market (1972 crop)	Composite of 50 leaves (5 leaves from each farmer)	0.35–4.0	Domanski et al., 1975 Gibson et al., 1974
Dark air cured tobacco	Auction market (1972 crop)	Composite of 50 leaves (5 leaves from each farmer)	0.36–1.69	Domanski et al., 1975; Gibson et al., 1974
Light air cured tobacco	Auction market (1972 crop)	Composite of 50 leaves (5 leaves from each farmer)	0.19	Domanski et al., 1975; Gibson et al., 1974
Cigarettes	Retail market	Individual samples of various brands were composited	3.3 in 1971 1.4 in 1973	Domanski et al., 1974
Cigars	Retail market	Individual samples of various brands were composited	<0.6 in 1971 0.3 in 1973	Domanski et al., 1974
Light cigars	Retail market	Individual samples of various brands were composited	<0.6 in 1971 0.1 in 1973	Domanski et al., 1974
Chewing tobacco	Retail market	Individual samples of various brands were composited	1.4 in 1971 0.5 in 1973	Domanski et al., 1974
Smoking tobacco	Retail market	Individual samples of various brands were composited	1.6 in 1971 1.0 in 1973	Domanski et al., 1974
Snuff	Retail market	Individual samples of various brands were composited	1.2 in 1971 0.6 in 1973	Domanski et al., 1974

1974). This suggests that toxaphene use with or near tobacco was greater in the past and is slowly declining.

4.3.2.2 Translocation -- A search of literature has revealed no experimental data concerning translocation of toxaphene in crop plants. It is believed that crop residues result primarily from surface deposition, and only little, if any, translocation of toxaphene occurs in plants (Hartwell et al., 1974). Accordingly, root crops will be expected to contain only traces of toxaphene.

4.3.2.3 Effects -- The effect of toxaphene on emergence, growth, physiological functions, and yield of various crop plants has been reported. In assessing the phytotoxic effect of insecticide treatment from field studies, the damage caused by the insecticide should be compensated for by the protection afforded by the insecticide. Such compensation, however, is not always easy to determine and, therefore, the results of such studies should be viewed with caution.

In an attempt to determine if accumulation of toxaphene in the soil might become detrimental to cotton, Franco et al. (1960) studied the effect of increasing soil concentrations of the insecticide on the growth of cotton plants. The studies were carried out in Mitscherlich pots in a greenhouse with two types of soil - one sandy and the other clay. In sandy soil, toxaphene added to the pot at a dose corresponding to the field dose of 72.3 kg/ha (calculated on area basis), when applied in emulsion form, showed some toxicity. However, the insecticide applied in powdered form equivalent to a field dose of 101.5 kg/ha did not exhibit any toxic effect. Toxaphene also failed to exert significant toxic effects in clay soil. Joyce (1954) reported spotting of leaves in cotton caused by toxaphene, but noted no prolonged effect on the plant and no subsequent deleterious effect on yield. The growth and development of cotton as affected by a toxaphene-DDT mixture (2:1) has been studied by Brown and coworkers (1961, 1962). Both yield and boll production were significantly increased in field plots upon treatment with toxaphene and DDT. No significant effect of the insecticide mixture was observed on internode length, number of internodes, plant height, percent of boll shed, germination, percent lint, boll components seed index, lint index, boll weight, and seeds per boll. The results suggest that toxaphene, as well as DDT, was relatively nontoxic to cotton.

Randolph et al. (1960) conducted field experiments to determine the effect of large amounts of toxaphene applied to soil on the germination, growth, and yield of several crops. The initial dosage was equivalent to the amount of toxaphene applied normally during 5 years for the control of cotton insects. Subsequently, normal foliar treatments were made annually to the crops grown in the treated plot. Cotton was grown immediately after application; sorghum 1 year after application; and alfalfa 2 years following cotton. No significant difference in the number of plants per acre and yield of the above crops was noted between the treatments and the control (Table 4.8). At the dosage of toxaphene equivalent to the amount that would be applied for control of insects in cotton over a 10 and 20-year period, adverse effects on some crops were observed. Most severely affected were the oat plants; the plant stand and yield of oat forage and grain were significantly less than those from untreated plots. The data must be considered, however, keeping in mind that the dose of toxaphene in these studies was several fold higher than normally recommended for insect control.

The effect of toxaphene on the growth and respiration of corn, oats, peas, and cucumber has been evaluated by Lichtenstein et al. (1962). The plants were

Table 4.8. Response of various crops to application of large amounts of toxaphene[a]

Rate and mode of application	Crops grown during 4-year period following application	Crop response examined		
		Plants/ha	Average height	Yield
			% of control	
A. 121.5 kg/ha preplanting application plus 14-19 lbs/acre annual foliar application	Cotton, immediately after application	112	102	117, seed cotton
	Sorghum, first year	88	---	106, forage; 103, grain
	Alfalfa, second year	102	---	---
	Alfalfa, third year	124	---	---
B. 165.3 kg/ha preplanting[b] application	Cotton, immediately after application	101	---	93, seed cotton
	Oats, first year	75	---	88, forage; 101, grain
	Cotton, third year	94	---	---
C. 309.4 kg/ha preplanting[c] application	Cotton, immediately after application	95	---	97, seed cotton
	Oats, first year	63	---	86, forage; 101, grain
	Cotton, third year	115	---	---

[a] Modified from Randolph et al., 1960

[b] Calculated amount that would be applied in 10 years.

[c] Calculated amount that would be applied in 20 years.

grown in quartz sand to keep sorption of the insecticide to a minimum. In general, toxaphene was toxic to these plants as revealed from stem and root length measurements, respiration of excised root tips, and dry weight per unit length of root tip (Table 4.9). A slight reduction over control was noted in peas in the ratio of root and stem length. However, considering that the conditions of treatment were extreme (for example, the quartz sand does not possess any insecticide-retention qualities), it is unlikely that toxaphene will be toxic to peas. In a greenhouse study, Hagley (1965) noted no significant effect of toxaphene at an application rate of 1.57 kg/ha on the growth and size of seedlings of cauliflower and tomato, but in cabbage a reduction in the size of the seedlings was observed (Table 4.10). At 15.7 kg/ha, the insecticide was found to reduce growth rate and the size of seedlings, but did not significantly affect root development. In some plants, toxaphene also caused marginal and interveinal chlorosis and necrosis of the lower leaves. The greatest phytotoxicity was observed in tomatoes resulting in the death of 50 and 33 percent of the seedlings in the second and third weeks of growth, respectively.

Probst and Everly (1957) observed no noticeable effect of toxaphene when applied as wettable powder in the soil on emergence, growth, yield, and chemical composition of soybeans. The chemical variables of soybean tested included protein, oil content, and iodine number of the oil. The dosage (44.8 kg/ha) used in these experiments was much greater than that recommended for the control of soil insects.

Considerable effort has been expended on the investigation of toxaphene as a possible cause of off-flavor in food products. Generally, researchers have attempted to answer three questions: (a) is the difference real; (b) is

Table 4.9. Effect of 30 ppm toxaphene, added at the time of planting, on growth and respiration of various crop plants[a]

Plant variable examined	Crop response in % of control plants			
	Corn	Peas	Oats	Cucumber
Stem length[b]	88	114	--	--
Root length[b]	87	108	--	--
Root length/stem length	1.02	0.67	--	--
Respiration of excised root tips[c]	99.9	83.9	103.6	116.9
Dry matter content of 10 mm root tip[c]	88	87	88	104

[a] Modified from Lichtenstein et al., 1962.
[b] 21 days after planting.
[c] 7 days after planting.

Table 4.10. Effect of toxaphene on the growth and size of vegetable seedlings[a]

Toxaphene dose (kg active ingredient/ha)	Plant growth variable studied	Crop response in percent of control plants		
		Cauliflower	Chinese cabbage	Tomato
1.5	Average weekly increase in size	107.0	---	80.5
	Average weight of stem and foliage	77.6	71.5[b]	87.6
	Average weight of roots	136.0	77.0	88.0
15.6	Average weekly increase in size	61.0[b]	---	50.6[b]
	Average weight of stem and foliage	77.6	69.5[b]	66.0[b]
	Average weight of roots	109.0	83.6	74.5

[a] Modified from Hagley, 1965.
[b] Significantly different from untreated mean.

the difference commercially important, and (c) is the difference good or bad. Kramer and Ditman (1956) reported no significant difference in the taste of four processed products - tomato juice, tomatoes, snap beans, and lima beans - which had been treated with a 2 percent toxaphene aerosol. Potatoes treated in a similar fashion showed an improvement in flavor. The information on the flavor change in this study was obtained by evaluating the taste scores of the judges by both the triangular (an example of an attributes method) and the variable method. Birdsall et al. (1957) studied the effect of toxaphene on flavors of vegetables grown in the presence of the insecticide applied by soil treatment and by foliar treatment. The rate of application of the insecticide (6.7 kg/ha on vegetable foliage, 26.9 kg/ha in soil) was four times the normal rate of application. No significant difference in taste was noted in treated and untreated raw and canned vegetables tested - beets, sauerkraut, carrots, onions, potatoes, squash, pumpkin, radishes, and cucumbers. Undesirable changes in flavor, however, occurred in cooked rutabagas, onions, and carrots. Application of toxaphene to the soil appears to cause more instances of changes in flavor than did applications to the foliage.

While many insecticides have been reported to cause an increase in the disaccharide content of rutabagas and red beets by stimulating sucrose synthesis, toxaphene was found to have no significant effect (Monnberg, 1959).

The ability of toxaphene to impart off-flavor to tobacco has been investigated by Townes et al (1958). The contamination of tobacco in several ways was examined, including: (a) build-up of soil residues; (b) uptake of insecticidal residues in tobacco grown in subsequent years; and (c) direct foliar application of tobacco. Following curing, the tobacco was given to cigarette companies which made it into unblended cigarettes [illegible] then submitted the cigarettes to taste-testing panels. No off-flavor in tobacco grown in fields receiving soil applications of toxaphene or in fields previously planted with cotton treated with toxaphene was noted (Table 4.11). Toxaphene, however, caused off-flavor when applied to tobacco foliage.

Table 4.11. Effect of application of toxaphene to tobacco on cigarette flavor[a]

Toxaphene formulation used	Mode of application	Application rate (kg/ha)	Report of panel
20% Toxaphene dust	Foliar	5.4	Objectionable
75% E.C.[b]	Foliar	2.1	Objectionable
10% Dust	Foliar	1.7-2.0	Objectionable
42% Strobane E.C.	Foliar	2.0	Objectionable
20% Toxaphene dust	Single application to soil	112.0	Satisfactory
20% Toxaphene	Eight applications to cotton foliage in preceeding year; tobacco grown in the same soil in succeeding year	78.4-112.0	Satisfactory
75% E.C.	Foliar application within 10 days of harvest	2	Objectionable

[a] Modified from Townes et al., 1958.
[b] E.C., emulsified concentrate.

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5.0 BIOLOGICAL ASPECTS IN WILD AND DOMESTIC ANIMALS

5.1 CHAPTER SUMMARY

Toxaphene has a broad spectrum of acute toxicity. It kills aquatic protozoans at levels as low as 7 ppt and can kill calves at oral doses of 50 mg/kg. Organisms at all levels have demonstrated the ability to bioconcentrate the pesticide, thus potentially passing on to the next higher trophic level a toxic or metabolically altered dose of toxicant.

5.1.1 Fish and Amphibians

The effects of toxaphene on fish and amphibians are important due to the position that fish occupy as the top consumers in most aquatic environments, and because of possible entry into other food webs including higher vertebrates and man.

Fish are able to absorb and bioconcentrate toxaphene in significant quantities. A large portion of this is complexed with the lipid stores. In young fish, large quantities of toxaphene are associated with the yolk sac. As the fish matures and loses its yolk sac, toxaphene levels decrease. Later, as the fish grows older and develops its own lipid stores, toxaphene levels again increase. Some species can concentrate up to 76,000 times the amount detectable in the surrounding water (Mayer et al., 1975). There is no evidence of toxaphene biotransformation in fish. Toxaphene residues remain in fish for many months, or up to several years, following toxaphene application to lakes. Prey fish appear to accumulate more than predator fish. Under identical conditions, prey fish - bluegills and suckers - accumulated 9.4 and 10.6 ppm, respectively, whereas predator fish - bass, northern pike, and walleyes - had body residues of 2.2, 3.3, and 1.2 ppm, respectively (Hughes and Lee, 1973).

Toxaphene causes increased calcium-to-collagen ratios in fish, resulting in increased fragility of the bones. Similarly, decreases in bone collagen amino acids such as alanine, valine, leucine, isoleucine, lysine, phenylalanine and hydroxyproline are prevalent. Decreased vitamin C content and a strong inhibition of ATPase activity occur in toxaphene-exposed fish (Mayer and Mehrle, 1975 a, b, 1976).

Because of its use as a pesticide, acute toxicity data are abundant (Section 5.2.2.2.1). Toxaphene is not selectively toxic to fish species yielding LC50 values ranging from 2 to 32 ppb. Compared to other pesticides, however, toxaphene is an extremely potent fish toxicant.

Subacute or chronic exposure to toxaphene results in growth depression, degenerative changes in the liver with a proliferation of rough endoplasmic reticulum, mitochondrial swelling and condensation of nuclear chromatin. Also noted are kidney necrosis, necrotic changes in the digestive tract and damage to the central nervous system.

Finally, fish species exposed to toxaphene have been reported to develop a resistance to the pesticide. Toxaphene-tolerant mosquito fish tested in Ferguson's laboratory (1964, 1965 a, 1969) had 6 to 48 times more resistance than fish without prior exposure to toxaphene. Later studies revealed that many species of fish can develop increased tolerances to toxaphene, and that certain populations had a genetic resistance to toxaphene.

5.1.2 Aquatic Invertebrates

Aquatic invertebrates from unicellular plankton species to oligocheates have been shown to bioaccumulate toxaphene. Indeed, toxaphene residues in many invertebrate plankton dwellers often exceed the original toxaphene application level for periods up to 9 months post-application (Johnson, 1966).

Toxaphene concentrations in excess of one ppm block egg development in clams, while 0.25 ppm toxaphene will kill 67% of the larvae. Most invertebrates develop hyperactivity, loss of equilibrium and coordination, paralysis, and death in response to toxic levels of toxaphene.

Sublethal doses of toxaphene decrease activity and slow down shell decomposition in those aquatic invertebrates which possess them.

An abundance of acute toxicity data exists. Wide ranges in lethal doses have been observed from the estuarine copepod, Acartia tonsa, with an LC50 (96 hr) of 7.20 ppt (Khattat and Farley, 1976) to the naiads of Pteronarcys california with an LC50 (24 hr) of 18.0 ppm.

Pathologically, acute exposure to toxaphene causes vacuolated granular cells in the hepatopancreas often times coupled with a contraction of the intestine. There is occasionally noted a sloughing of the stomach epithelium, irregular spaces in the nervous system, and the deposition of an undefined orange-brown pigment in the body spaces (Courtenay and Roberts, 1973).

Chronic exposure to toxaphene results in decreased weight, tissue changes in the kidney, gills, and digestive tubules, and the invasion of the body cavity by a mycelial fungus (Lowe et al., 1971).

Many species have developed a resistance to toxaphene. In areas of high toxaphene use, freshwater shrimp have a toxaphene tolerance 3.5 times greater than non-exposed shrimp (Naqvi and Ferguson, 1970). As with fish, the ability to bioaccumulate large quantities of toxaphene has implications that extend to higher trophic levels.

5.1.3 Birds and Terrestrial Wildlife

No information is available on absorption, transport and distribution, biotransformation and elimination in birds and terrestrial wildlife.

Residues in birds are well documented. Residue levels in birds appear to be indicative of duration of exposure, exposure level, and amount of adipose tissue.

At the biochemical level, toxaphene has been shown to increase thyroid weight along with a possible stimulation of iodine incorporation.

Acute toxicity studies have yielded a variety of LC50's in a wide range of species. These values range from 50 to 316 mg/kg body weight depending on age, sex, route of administration, etc., and are summarized in Table 5.39.

Chronic toxicity data are limited. Pathological changes in pelicans fed toxaphene-treated sardines included a decrease in the subcutaneous and mesintery fat deposits (Keith, 1965). Ring-necked pheasants survived three months of 300 ppm toxaphene treatment with only a transitory weight loss and an increased vacuolation of liver tissue (Genelly and Rudd, 1956 a). However, quail exposed to 0.1 percent toxaphene experienced 100 percent mortality in 13 days (Linduska and Springer, 1951).

Toxaphene, at high dietary doses, has also been shown to decrease egg laying, egg hatchability, and post-hatching survival. These factors, coupled with the bioconcentration factor many birds experience in their diet, could have profound effects on the survival of susceptible bird species.

5.1.4 Domestic Animals

The metabolic data which are presently available deal only with toxaphene tissue residues. Toxaphene use to control ectoparasites has led to the appearance of residues in the meat and milk of these animals.

Young calves are susceptible to acute toxaphene poisoning when applied as a spray or dip. Death has resulted from application levels as low as one percent.

Chronic exposure data are rather limited. Animals fed toxaphene-treated hay experienced transitory central nervous system effects and no other overt signs of toxicity. Toxaphene has also been shown to depress rumen function.

The potential for contamination of meat and milk with toxaphene should be adequately monitored so as to protect man from excessive exposure.

5.1.5 Terrestrial Insects and Other Terrestrial Invertebrates

Metabolically, toxaphene has been shown to penetrate the hemolymph and to bind with the nervous system tissue. Physiologically, toxaphene appears to alter central nervous system activity and neuron ion permeability. In the house fly, toxaphene alters the metabolism of formate by increasing uric acid and decreasing proline levels.

Toxaphene has been widely used for the protection of cotton crops from lepidoptern pests for over 20 years. Acute toxicity data for these pests and other insects are presented in Table 5.59.

Resistance to toxaphene has been demonstrated in several orders of insects. Resistant insect strains can tolerate doses of toxaphene ten times greater than those causing death in susceptible strains. In attempts to explain this developed or genetic resistance, Reiser et al. (1953) found that in boll weevils the survivors had 10 percent more fat per unit body weight. Moore et al. (1967) correlated survival with higher levels of palmitic and oleic acids. Moore and Taft (1972) found higher levels of triglycerides in the survivors. The development of completely resistant strains of pest organisms is not a unique problem associated with toxaphene, but one which should be continuously monitored.

5.2 FISH AND AMPHIBIANS

Because of the general interest in fish as an environmental resource and the use of toxaphene as a piscicide, extensive information is available on the effects of toxaphene on fish. This information is presented in the appropriate sections below. Amphibians have received much less attention, and all of the information on these animals is presented in Section 5.2.2.2.8.

5.2.1 Metabolism

5.2.1.1 Absorption/Bioconcentration -- Relatively few controlled studies have been conducted on the absorption of toxaphene by fish. While the toxaphene residue studies described in Section 5.2.1.5 indicate that fish are able to absorb toxaphene directly from the water and/or from contaminated fish food organisms, such studies do not include sufficient details on exposure conditions and the effects of toxaphene metabolism to justify quantitative statements on toxaphene absorption and bioconcentration. Nevertheless, given the information described below, fish seem able to rapidly bioaccumulate toxaphene from water and to bioconcentrate this compound by a factor of several thousand.

The short term uptake kinetics of Cl-36 labelled toxaphene in mosquito fish have been examined by Schaper and Crowder (1976). In this study, fish were exposed to 2 ppm toxaphene in water for eight hours, and toxaphene uptake in whole fish was determined at one-hour intervals. As indicated in Figure 5.1, absorption was linear under these conditions with detectable concentrations of toxaphene appearing in whole fish after one hour. After eight hours, toxaphene content in whole fish averaged 0.586 micrograms/gram (ppm), thus indicating that bioconcentration did not occur during this relatively brief exposure period.

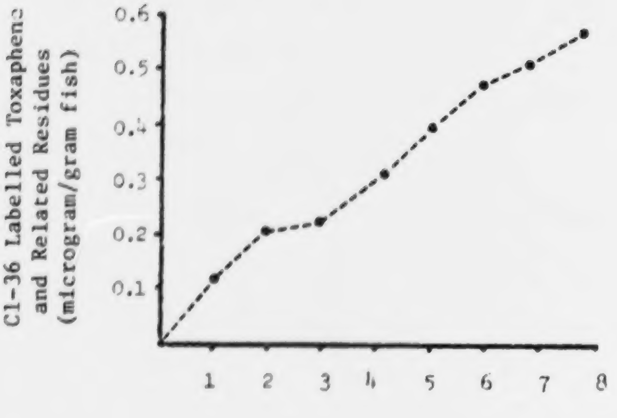


Figure 5.1. Uptake of Cl-36 labelled toxaphene and toxaphene related residues in mosquito fish exposed to 2 ppm Cl-36 toxaphene. Source: Schaper and Crowder, 1976.

During longer exposure periods to markedly lower levels of toxaphene, bioconcentration of toxaphene has been demonstrated in both bluegills and brook trout. In static 96-hour exposures to toxaphene concentrations of 17.3 ppb and 70.3 ppb, bluegills accumulated mean whole body concentrations of 9.4 ppm and 7.7 ppm, respectively (Hughes, 1970). However, the differences between these means were not statistically significant at p less than 0.05, suggesting that at both test concentrations the mechanisms of toxaphene absorption were saturated. At toxaphene concentrations of 0.5 ppb and below, Mayer and coworkers (1975) have shown that in brook trout both the amount of toxaphene absorbed and the extent of bioconcentration are generally directly proportional to the concentration of toxaphene in the water. However, the time-course of toxaphene absorption and bioconcentration is somewhat complex. As indicated in Table 5.1, brook trout fry continuously exposed to toxaphene at concentrations of 0.041 ppb to 0.500 ppb concentrated toxaphene from the water by a factor of up to 76,000. Maximum whole-body residue and maximum bioconcentration at all exposure levels occurred after 15 days, declined between days 15 and 60, then increased up to day 90. The extraordinarily high levels of toxaphene at day 15 were partially attributed to the presence of the yolk sac which contains large quantities of lipids, and thus might be expected to store appreciable amounts of toxaphene. The decrease in tissue residue and bioconcentration by day 60 could correspond to the disappearance of the yolk sac and a relatively low level of body lipids. The increased body levels of toxaphene by day 90 are most likely attributable to the increasing lipid content of maturing fry (Mayer et al., 1975).

Yearling brook trout seem to bioconcentrate toxaphene less extensively than do trout fry (Table 5.2). During 161 day exposure periods, these yearlings

Table 5.1. Whole-body residues of toxaphene in brook trout fry continuously exposed to toxaphene[a]

Exposure level	Statistic	Days of exposure			
(ppb)		7	15	60	90
0.041[b]	Residue[d]	0.2	2.6	0.4	0.6
(.039 ± .003)[c]	Number	2	2	2	2
	BCF[e]	4,900	63,400	9,800	14,600
0.075	Residue	1.0	3.7	0.9	1.4
(.068 ± .004)	Number	2	2	2	2
	BCF	13,300	49,300	12,000	18,700
0.125	Residue	2.2	8.3	1.8	2.6
(.139 ± .010)	Number	2	2	2	2
	BCF	17,600	66,400	14,400	20,800
0.270	Residue	4.5	18	-[f]	-
(.288 ± .022)	Number	2	2	-	-
	BCF	16,700	66,700	-	-
0.500	Residue	9.2	38	-	-
(.502 ± .040)	Number	2	2	-	-
	BCF	18,400	76,000	-	-

[a] Source: Modified from Mayer et al., 1975.

[b] Nominal concentration of toxaphene in water (micrograms/liter).

[c] Measured concentration of toxaphene in water ± the standard error (micrograms/liter).

[d] Whole-body toxaphene residue from composite sample of several fry (2 g) (micrograms/gram).

[e] Bioconcentration factor: toxaphene residue in whole-body samples ÷ toxaphene concentration in water.

[f] All fish were dead.

Table 5.2. Whole-body residues of toxaphene in yearling brook trout continuously exposed to toxaphene[a]

Exposure level	Statistic	Days of exposure							
(ppb)		3	7	10	18	24	60	140	161
0.041[b]	Residue[d] (ppm)	N.D.[f]		N.D.		< 0.1	< 0.1	0.3 ± 0.005	0.2 ± 0.03
(.039 ± .003)[c]	Number	4		4		4	4	4	4
	BCF[e]	-		-		< 2,400	< 2,400	7,300	4,900
0.075	Residue		< 0.1		< 0.1		0.1 ± 0.02	0.8 ± 0.08	0.4 ± 0.04
(.068 ± .004)	Number		4		4		6	6	7
	BCF		< 1,300		< 1,300		1,300	10,700	5,300
0.125	Residue	N.D.		< 0.1		0.3 ± 0.11	0.5 ± 0.03	0.6 ± 0.06	0.4 ± 0.06
(.139 ± .010)	Number	4		4		4	4	4	4
	BCF	-		< 800		2,400	4,000	4,800	3,200
0.270	Residue		< 0.1		0.3 ± 0.06		1.8 ± 0.12	3.5 ± 0.30	2.4 ± 0.19
(.288 ± 22)	Number		4		4		6	5	8
	BCF		< 370		1,100		6,700	13,000	8,900
0.500	Residue	< 0.1		0.5 ± 0.04		2.0 ± 0.12	4.5 ± 0.46	7.7 ± 0.10	8.0 ± 0.55
(.502 ± .040)	Number	4		4		4	4	4	3
	BCF	< 200		1,000		4,000	9,000	15,400	16,000

[a] Source: Modified from Mayer et al., 1975.
[b] Nominal concentration of toxaphene in water (micrograms/liter).
[c] Measured concentration of toxaphene in water ± the standard error (micrograms/liter).
[d] Whole-body toxaphene residue ± the standard error in micrograms/gram.
[e] Bioconcentration factor: body residue ÷ toxaphene concentration in water.
[f] None detected, detection limit 0.05 micrograms/gram.

concentrated toxaphene from the water by factors of from 3,200 to 16,000. As with the trout fry, the extent of toxaphene bioconcentration at a given time was generally proportional to exposure concentrations, although fish in the 0.125 ppb exposure group concentrated less toxaphene than would be expected after 60 days. At all exposure levels below 0.500 ppb, maximum toxaphene bioconcentration occurred on or after day 140 of exposure and declined appreciably by day 161. The reasons for this decline were not examined.

The transfer of toxaphene to fish from fish food organisms has received little study. As described in Section 5.3.1, Schoettger and Olive (1961) found that fish died when fed on Daphnia which had been exposed previously to sublethal levels of toxaphene. However, these investigators did not determine if lethality was attributable directly to the ingestion of toxaphene in contaminated Daphnia or to leaching of toxaphene from the Daphnia with subsequent reabsorption by the fish. Recently, Sanborn and coworkers (1976) have followed the fate of C-14 labelled toxaphene in a terrestrial-aquatic model ecosystem. At a concentration of approximately 44.4 ppb toxaphene in water, mosquito fish concentrated toxaphene by a factor of 4,247 over a three-day exposure period.

5.2.1.2 Transport and Distribution -- As with the pattern seen in mammals (Section 6.1.2), the limited available information on fish suggests that fat tissue is a primary site of toxaphene storage. In bluegills exposed to toxaphene in toxaphene-treated lakes for periods of up to 350 days, a higher correlation was found between toxaphene concentration and fat content in whole fish, fish fillets, and fish liver than between toxaphene content and whole animal or organ weight. Further, the edible portion of the fish (fillets) contained only one-sixth the concentration of toxaphene found in whole fish and only about one-half the concentration of toxaphene found in liver (Hughes, 1970; Hughes and Lee, 1973). Similar results have been found in 161 day exposures of adult brook trout to concentrations of toxaphene ranging from 0.041 ppb to 0.500 ppb (Mayer et al., 1975). Toxaphene residues found in trout fillet were about one-third to one-fourth of those found in the remaining tissue (Table 5.3).

5.2.1.3 Biotransformation -- The ability of fish to metabolize toxaphene cannot be determined from the available data. In 161 day exposures of brook trout to 2 ppm toxaphene, Mayer and coworkers (1975) noted that GLC peak patterns of toxaphene tissue residues differed from those of toxaphene standards. For the most part, these differences indicated preferential tissue storage of the more highly chlorinated lipophilic toxaphene isomers. Similar alterations in chromatographic patterns have been noted in fish taken from toxaphene-treated lakes (Hughes, 1970; Hughes and Lee, 1973; Terriere et al., 1966). While these results would certainly be expected if fish significantly metabolized various toxaphene components, such alterations in peak patterns also could be explained on the basis of preferential absorption or elimination without actual biotransformation. Further, because toxaphene is known to be altered in aquatic systems (Section 7.3.2), altered peak patterns in fish taken from toxaphene-treated lakes do not necessarily indicate that toxaphene is metabolized by fish.

Schaper and Crowder (1976) have found no evidence that mosquito fish are able to metabolize toxaphene. After eight hours exposure of these fish to 2 ppm Cl-36 labelled toxaphene, virtually all of the recoverable label was found in the nonpolar fraction (hexane) and none in the polar fraction (water). While these results do not rule out the possibility of minor metabolic conversions, they do suggest that substantial dechlorination or conversion of toxaphene to water soluble metabolites did not occur.

Table 5.3. Toxaphene residues found in adult brook trout fillet and remaining tissue after 161 days of exposure[a]

Exposure, ppb	Statistic	Fillet	Offal
0.041[b]	Mean ± S.E.[c]	< 0.10	0.42 ± 0.05
	Number	4	4
0.075	Mean ± S.E.	0.17 ± 0.04	0.62 ± 0.07
	Number	4	4
0.125	Mean ± S.E.	0.21 ± 0.3	0.66 ± 0.11
	Number	4	4
0.270	Mean ± S.E.	0.87 ± 0.13	3.0 ± 0.2
	Number	4	4
0.500	Mean ± S.E.	4.0 ± 0.72	11 ± 1.2
	Number	3	3

[a] Source: Mayer et al., 1975.
[b] Nominal concentration.
[c] Standard error.

5.2.1.4 Elimination -- The elimination of toxaphene by fish seems to depend, at least in part, on the total body burden of toxaphene. In a follow-up to the absorption studies described in Section 5.2.1.1, Mayer and coworkers (1975) placed adult brook trout, which had been previously exposed to known levels of toxaphene, into toxaphene-free water and monitored the decrease in total body residue over a 56 day period (Table 5.4). Only fish from the two higher exposure concentrations, 0.27 ppb and 0.5 ppb, eliminated detectable quantities of toxaphene, 32 percent and 51 percent, respectively. As indicated in the previous section, the lower chlorinated toxaphene isomers appeared to be eliminated more readily than the more highly chlorinated isomers based on shifts in chromatographic patterns of toxaphene residues. Trout in the 0.075 ppb and 0.125 ppb exposure groups released low, but consistent, levels of toxaphene throughout the 56 day depuration period.

The failure to detect toxaphene in fish from the 0.041 ppb exposure group after 28 and 56 days may be attributable to the analytical technique which was not quantitatively valid below residue levels of 0.1 ppm and had a minimum detection limit of 0.05 ppm (Mayer et al., 1975). Schaper and Crowder (1976) were also unable to detect toxaphene elimination in mosquito fish during a six-hour period after exposure to 2 ppm toxaphene for eight hours. The total body residue in these fish, 0.6 ppm, approximated that of the 0.125 ppb exposure group in the study by Mayer and coworkers (1975).

5.2.1.5 Residues -- Studies on toxaphene residues in fish fall into two basic groups. The first involves periodic monitoring in lakes which had been treated with toxaphene as part of fish eradication programs. Such studies are useful in that they often provide some indication of the duration and extent of toxaphene

Table 5.4. Toxaphene residues in adult brook trout after transfer to uncontaminated water[a]

Exposure[b] ng/l	Statistic	(ppm)			
		Days after cessation of toxaphene exposure			
		7	14	28	56
0.041[c]	Mean ± S.E.[d]	0.1 ± 0.03	0.1	N.D.[e]	N.D.
	Number	3	2	2	2
0.075	Mean ± S.E.	0.3 ± 0.00	0.2 ± 0.13	0.4 ± 0.10	0.3 ± 0.06
	Number	3	3	3	3
0.125	Mean ± S.E.	0.7	0.8	0.9	0.7 ± 0.03
	Number	2	2	2	3
0.270	Mean ± S.E.	2.2 ± 0.17	1.6 ± 0.10	1.4 ± 0.35	1.5 ± 0.07
	Number	3	3	3	3
0.500	Mean ± S.E.	7.5 ± 0.62	5.8 ± 0.59	5.8 ± 0.58	3.7
	Number	3	3	3	2

[a] Source: Mayer et al., 1975.
[b] Toxaphene concentration in water before cessation of toxaphene exposure.
[c] Nominal concentration.
[d] Standard error.
[e] None detected, detection limit 0.05 ppm.

exposure, as well as information on the kinetics of toxaphene uptake and species specific differences in toxaphene bioconcentration. The second type of study usually involves one-time measurements of toxaphene fish residues in aquatic systems not specifically treated with toxaphene. These studies presumably give some indication of ambient levels of fish contamination by toxaphene.

Toxaphene residues found in fish from toxaphene-treated lakes are generally consistent with the results of laboratory studies described previously and indicate that fish bioconcentrate toxaphene by a factor of several thousand. Kallman and coworkers (1962) monitored the uptake of toxaphene by rainbow trout and bullheads in Clayton Lake, New Mexico. Seventy-two hours after the initial application of toxaphene, toxaphene water levels ranged from undetectable (limits of detection not given) to 12 ppb. Assuming zero values for all samples in which toxaphene was not detected, the average water concentration for this period was 2.4 ppb. Over this period, trout concentrated toxaphene by factors of 180 to 2250 having toxaphene residues of 0.43 ppm to 5.4 ppm. Over the same period, bullheads concentrated toxaphene by factors of 200 to 1750 with toxaphene residues ranging from 0.51 ppm to 4.2 ppm. Five days after initial treatment, average toxaphene water concentration was 12 ppb (2 ppb on the windward side and 21 ppb on the lee side). By this time, bullheads were severely affected by the toxaphene and had mean body residues of about 13 ppm. After toxaphene water levels in the lake stabilized at about 1 ppb, trout exposed for seven days had toxaphene whole-body residues between 0.8 ppm and 2.5 ppm (bioconcentration factors of 800 to 2,500). In similar exposures for 17 days, trout bioconcentrated toxaphene by factors of 1,300 to 3,500. By 523 days after initial toxaphene treatment, no toxaphene was detected in the water, but trout exposed for 108 to 170 days had body residues of 0.3 ppm.

In a similar study, Terriere and coworkers (1966) monitored toxaphene water levels and fish residues in two Oregon Lakes: Davis Lake treated in the fall of 1961 at a calculated concentration of 88 ppb and Miller Lake treated in the fall of 1958 at a calculated concentration of 40 ppb. Results for Davis Lake are summarized in Table 5.5. Rainbow trout consistently accumulated greater levels of toxaphene than Atlantic salmon. As in the experimental exposures by Mayer and coworkers (1975), fish concentrated the toxaphene by a factor of several thousand. For Miller Lake, residue levels were determined only in brook trout for one year, 1964. Here, toxaphene water concentration was 0.84 (0.7 to 1.1) ppb and whole body residues in brook trout were 12.46 (8.30 to 24.80) ppm with a bioconcentration factor of about 14,800. The rate of toxaphene uptake by brook trout in Miller Lake over 10 to 18 day periods was determined by suspending the fish in live boxes for varying periods of time (Figure 5.2). As in the short-term uptake studies by Schaper and Crowder (1976), uptake appeared to be linear over the first ten days but plateaued after this time in the 1964 test.

Hughes and Lee (1973) present long term kinetic data on toxaphene residues in bluegills from Fox Lake (Figure 5.3). Two groups of fish were monitored in this study: those that had been placed into the lake 11 months after initial toxaphene treatment (stocked), and young bluegills from the successful spawning of this first group (hatched). At all times during this study, toxaphene concentration in the water was below detectable limits (1 ppb). However, the peak concentration at 200 days after stocking in both groups of fish is presumed to represent the time that toxaphene water concentration became so low that water-tissue equilibrium reversed in favor of toxaphene elimination. It is interesting to note that after 787 days, toxaphene residues in both groups

Table 5.5. Toxaphene total body residues in fish from Davis Lake[a]

	Year		
	1962	1963	1964
Toxaphene concentration in water, ppb[b]	0.63 (0.5-0.9)	0.41 (0.3-0.6)	< 0.2
Rainbow trout			
Residue, ppm	5.7 (1.2-12.0)	7.72 (2.75-13.7)	3.5 (3.2-3.8)
BCF[c]	9,050	18,830	> 17,500
Atlantic salmon			
Residue, ppm	2.75 (2.6-2.9)	3.24 (1.11-5.50)	1.8 (1.5-2.1)
BCF[c]	4,370	7,900	> 9,000

[a] Source: Terriere et al., 1966.

[b] All figures give mean value and range in parentheses.

[c] Bioconcentration factor: average tissue residue ÷ average concentration of toxaphene in water.

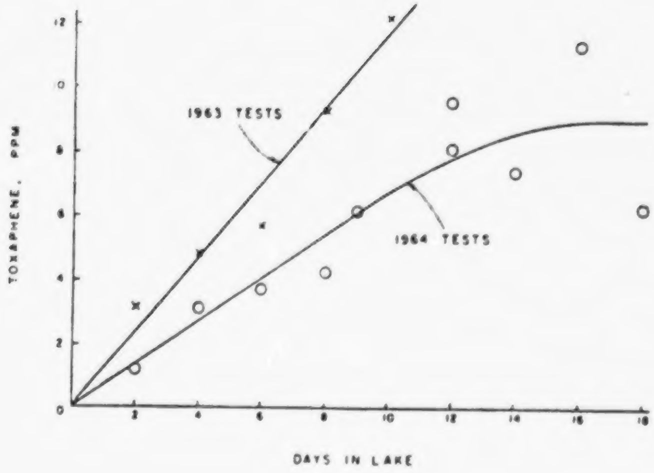


Figure 5.2. The rate of uptake of whole-body levels of toxaphene by rainbow trout in Miller Lake. Average concentration of toxaphene in water was 1.20 ppb for 1963 and 0.84 ppb for 1964. Source: Terriere et al., 1966.

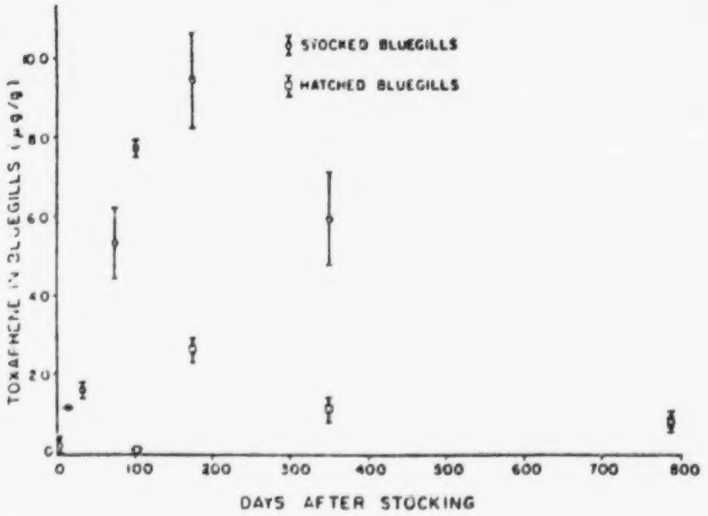


Figure 5.3. Variation, with time, of toxaphene concentration in bluegills from Fox Lake, mean and range indicated.
Source: Hughes and Lee, 1973.

of fish approached 1.0 ppm which is about the concentration at which Mayer and coworkers (1975) were able to demonstrate no significant elimination after 53 days (Section 5.2.1.4). In another phase of this study, Hughes and Lee (1973) found that predator fish accumulated less toxaphene than prey fish. Six months after stocking, bluegills and suckers, both prey fish, had total body residues of 9.4 ppm and 10.6 ppm, respectively. At the same time, the predator fish bass, northern pike, and walleyes had body residues of only 2.2 ppm, 3.3 ppm, and 1.2 ppm, respectively.

Studies giving toxaphene residues in fish from aquatic systems not specifically treated with toxaphene are summarized in Table 5.6. Some of these studies reenforce various aspects of toxaphene metabolism previously described. This is particularly true with reference to the distribution of toxaphene described in Section 5.2.1.2. The results of Grzenda and Nicholson (1965) suggest that, in most species studied, the edible portion of the fish tends to accumulate less toxaphene than offal. The presence of toxaphene at 50 ppm in carp fat as compared to only 0.44 ppm in the gills is consistent with the supposition that toxaphene is preferentially sequestered in fat tissue (Johnson and Lew, 1970).

Two recent monitoring surveys failed to find toxaphene in fish samples. Reimold and Shealy (1976) monitored pesticide residues in fish collected from eleven estuaries representing all the Atlantic drainage basins in Georgia and South Carolina. The surveys were conducted semi-annually from 1972 to 1974. Although dieldrin, DDT, PBC's and mercury were found in many samples, toxaphene was not detected. Similarly, toxaphene was not found in fish taken from a New Jersey salt marsh in 1967 and 1973 (Klaas and Belisle, 1977).

Table 5.6. Toxaphene residues in fish from various fresh-water systems

Fish	Location	Tissue	Residue (ppm) (average range)	Reference
Rainbow trout	New Mexico (lake)	Whole body	0.06	Kallman et al., 1962
Bullhead	" "	" "	0.41	
White sucker	South Dakota (lake)	Whole body minus internal organs	0.083	Hannon et al., 1970
Carp	" "		0.176	
Northern pike	" "		0.074	
Catfish	Mississippi, commercial fish ponds	Fillet	1.98 (0.0-20.67)	Hawthorne et al., 1974
Carp	Colorado River Basin	Fat	50.0	Johnson and Lew, 1970
		Gills	0.44	
Channel catfish	" "	Muscle	6.8	
		Red muscle	0.55	
		Scraped skin	1.32	
		Fat	9.8 (8.2-11.38)	
Threadfin shad	" "	Whole fish	1.05	
		Skin and muscle	1.73	
		Gills	4.75	
		Ovary	0.70	
		Visera	1.71	
Sonoran sucker	" "	Skin and muscle	2.19 (0.0-0.25)	
		Kidneys	4.23	
		Viscera	32.52 (2.75-172.92)	
Gilia sucker	" "	Whole fish	25.00	
		Skin and muscle	2.81 (0.71-4.91)	
		Viscera	34.09 (25.24-42.94)	

Table 5.6 (continued)

Fish	Location	Tissue	Residue (ppm) (average range)	Reference
Chain pickerel	Maine	Whole-body	0.36	Henderson et al., 1969
White sucker	Delaware River	" "	1.60	
White perch	Lake Ontario	" "	1.25	
Fresh water drum	Lake Erie	" "	.01, 0.24	
Spotted sucker	South Carolina	" "	0.03	
Carp	Louisiana Arizona	" " " "	0.03 0.01	
Smallmouth buffalo	Arkansas	" "	0.01, 0.02	
Largemouth bass	Oklahoma	" "	0.01	
Channel catfish	Utah	" "	0.01	
Green sunfish	Alabama Basin	Fillet Offal	0.34 0.55	
Largemouth bass	" "	Fillet Offal	0.80 3.30	Grzenda and Nicholson, 1965
Redhorse suckers	" "	Fillet Offal	0.99 2.10	
Green chubs	" "	Fillet Offal	0.96 2.90	
Gizzard shad	" "	Fillet Offal	1.32 1.70	
Warmouth bass	" "	Fillet Offal	0.30 0.71	

Table 5.6 (continued)

Fish	Location	Tissue	Residue (ppm) (average range)	Reference
Largemouth bass	California	Flesh	0.05 (0.0-0.3)	Keith and Hunt, 1966
		Viscera	1.13 (0.2-2.0	
Brown bullhead	" "	Flesh	0.06 (0.0-0.19)	
Carp	" "	Flesh	0.01	
		Viscera	0.05 (0.0-0.1)	
Channel catfish	" "	Fat	0.4	
Black crappie	" "	Whole-body	0.03 (0.0-0.1)	
Tui chub	" "	Whole-body	1.09 (0.0-8.0)	
Pumpkinseed	" "	" "	0.04	
Rainbow trout	" "	Flesh	0.22 (0.0-2.57)	
Bluegill	Louisiana	Whole-body	0.48 (0.0-2.06)	Epps et al., 1967
Channel catfish	" "	" "	2.23 (0.0-6.6)	
Gizzard shad	" "	" "	1.49 (0.0-4.75)	

5.2.2 Effects

5.2.2.1 Physiological and Biochemical Effects -- Chronic toxaphene exposure causes biochemical alterations associated with the "broken back" syndrome in fathead minnows, brook trout, and channel catfish (Mayer et al., 1975; Mayer and Mehrle, 1976; Mehrle and Mayer, 1975 a,b). Clinical details of this syndrome are discussed in Section 5.2.2.2.2. Fathead minnows exposed to toxaphene concentrations ranging from 0.055 ppb to 0.621 ppb for 150 days had significantly increased levels of calcium but decreased levels of collagen in the backbone (Table 5.7). Phosphorous levels, however, were not affected. Because collagen is the primary constituent of the organic matrix around which calcification and mineralization occur, Mehrle and Mayer (1975 a) proposed that the increased calcium to collagen ratio made the backbones of these fish more fragile and more liable to fracture. As detailed in Section 5.2.2.2.2, electrical shock experiments support this conclusion. In addition to the effects on collagen and calcium, all exposures caused significant (p less than 0.05) decreases in the following amino acids of bone collagen: alanine, valine, leucine, isoleucine, lysine, phenylalanine, and hydroxyproline. In the skin of exposed fish, only hydroxyproline content decreased. However, because this amino acid is important in the formation of collagen, which is in turn essential for wound healing, this effect may further limit the ability of fish to cope with environmental stress.

Using hydroxyproline as an index of collagen synthesis, Mehrle and Mayer (1975 b) have demonstrated that toxaphene concentrations as low as 0.068 ppb inhibit collagen synthesis in brook trout sac fry by seven days after hatching. Similar to the results with minnows, continuous exposure to low level toxaphene concentrations for up to 90 days caused a pronounced decrease in collagen content and somewhat less pronounced increases in calcium content in the backbones

Table 5.7. Collagen, calcium, and phosphorus concentrations in fathead minnow backbones as affected by 150 day continuous exposures to various levels of toxaphene[a]

Backbone constituent	Toxaphene concentration (ppb)					F value[b]	$LSD_{.05}$
	0	0.005	0.132	0.288	0.621		
Collagen	32.27	26.89[c]	22.92[c]	19.94[c]	22.39[c]	7.64	2.14
Calcium	11.81	15.10[c]	23.26[c]	20.84[c]	24.31[c]	3.27	2.85
Phosphorus	4.08	3.55	3.66	3.78	3.48	2.63	0.19

[a] Source: Mehrle and Mayer, 1975 a.
[b] $F_{0.05} = 2.64$; $F_{0.01} = 3.91$.
[c] Significantly different from controls ($P < 0.05$).

of these fish (Table 5.8). Because proline hydroxylase (the enzyme responsible for the conversion of proline residues to hydroxyproline residues) requires ascorbic acid as a cofactor, Mehrle and Mayer (1975 a,b) proposed that a local vitamin C deficiency might account for these biochemical effects. Recently, Mayer and Mehrle (1976) did show that decreased collagen content in the backbones of channel catfish was accompanied by decreased backbone vitamin C content after toxaphene exposures similar to those described above. In that liver vitamin C was not affected, these investigators suggest toxaphene induces hepatic microsomal hydroxylases - e.g., P-450 - which utilizes vitamin C and thus causes a shift of vitamin C from the backbone to the liver.

Like many chlorinated pesticides, toxaphene exerts a strong inhibitory effect on fish ATPase activity. In vitro, toxaphene at 40 ppm inhibited NaKMg-ATPase, NaK-ATPase, and MgATPase activities in trout gill microsomes by 49 percent, 36 percent, and 66 percent, respectively. At 4 ppm, only NaKMg-ATPase (8.6 percent inhibition) and Mg-ATPase (24 percent inhibition) were significantly affected (Davis et al., 1972). Similar inhibition has been demonstrated in three types of ATPase activity in catfish brain, kidney, and gill (Table 5.9). Oligomycin insensitive (i.e., non-mitochondrial) Mg-ATPase was the most severely inhibited ATPase in all tissue. For the most part, brain and gill ATPases were more severely affected than kidney ATPases (Desaiah and Koch, 1975).

Toxaphene, along with a number of other chlorinated and organophosphate insecticides, has also been shown to inhibit oxygen uptake by bluegill liver mitochondria. At a concentration of 0.41 mg toxaphene per ml of reaction medium, mitochondrial oxygen uptake was depressed by 69 percent. No significant effect, however, was seen on phosphate uptake (Hiltibran, 1974). The

Table 5.8. Backbone composition of brook trout fry exposed to toxaphene.[a] Mean values expressed on basis of dried weight of backbone

Days after hatching, and backbone constituent[b]	Toxaphene concn. (ppb)				
	0	0.039	0.068	0.139	0.288
30 days					
Collagen[c] (mg HyP/g)	51.34	34.66^{d}	26.16^{d}	19.76^{d}	24.99^{d}
Phosphorus (%)	5.73	7.30	9.89	8.80	10.90
Calcium (%)	1.23	0.98	1.33	1.05	1.43
60 days					
Collagen (mg HyP/g)	20.10	13.86^{d}	14.02^{d}	10.98^{d}	-^{e}
Phosphorus (%)	12.05	10.81	9.89	7.71^{d}	-
Calcium (%)	2.64	2.85	2.54	1.31	-
90 days					
Collagen (mg HyP/g)	19.48	16.42^{d}	16.22^{d}	16.42^{d}	-
Phosphorus (%)	10.60	15.61	20.25^{d}	19.87^{d}	-
Calcium (%)	10.20	15.34	20.61^{d}	21.14^{d}	-

[a] Source: Mehrle and Mayer, 1975 b.

[b] n = 6-8.

[c] Collagen estimated by determination of hydroxyproline.

[d] Significantly different from controls ($LSD_{0.05}$).

[e] All fish exposed to 288 ppb toxaphene died within 60 days and all those exposed to 0.502 ppb (not shown) died within 30 days.

Table 5.9. Inhibition of catfish brain, kidney, and gill ATPases by toxaphene[a]

Toxaphene (micromoles)				% Inhibition[b]					
	NaK-ATPase			Oligomycin sensitive Ms-ATPase			Oligomycin insensitive Mg-ATPase		
	Brain	Kidney	Gill	Brain	Kidney	Gill	Brain	Kidney	Gill
1.8	8.2	-8.4[c]	-2.0	19.3	-49.7	-6.7	29.7*	1.5	27.6*
3.6	25.9	29.5	22.2	24.5	- 8.5	-2.5	42.5**	16.0	33.9*
7.2	41.3*	31.6	35.4*	46.1*	-3.1	9.0	49.9***	28.0*	47.8**
14.5	48.0**	33.9	36.1*	46.1	22.9	40.9**	72.0***	36.2**	56.6***
29.0	50.0**	33.7	37.4*	59.3*	42.3	50.9***	74.8***	39.7**	60.5***

[a] Source: Modified from Desaiah and Koch, 1975.
[b] Statistically significant when determined by Student's t test, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
[c] Negative (-) values indicate per cent stimulation of enzyme activity.

inhibition of oxygen uptake is consistent with the signs of respiratory distress seen in toxaphene-poisoned fish (Section 5.2.2.2.1).

5.2.2.2 Toxicity

5.2.2.2.1 Acute toxicity -- Because toxaphene has been used as a piscicide in fish management programs (Section 5.2.2.2.5), many studies have been conducted on the acute toxicity of toxaphene to fish. In that such fish management programs have been directed towards the elimination of rough fish in favor of game fish, several of these studies have attempted to determine if toxaphene is selectively toxic to certain families or species of fish. As summarized in Table 5.10, such selective toxicity does not appear to exist. Of the eight families of fish for which acute toxicity estimates are available, the 96-hour LC50's range from 2 ppb to 32 ppb. However, no consistent differences are apparent among the various families of fish. For example, the salmonidae, a family consisting largely of desirable game fish, has 96-hour LC50 values ranging from 2.5 ppb to 11 ppb. The ictalurids and cyprinids, which are less regarded as game fish, have comparable LC50 ranges of 2.7 to 13 ppb and 4 to 32 ppb, respectively. Such a comparison, based on the combined results of different investigators using a variety of different conditions and techniques, may be of limited validity. As discussed in the following paragraphs, such factors as pH, temperature, and alkalinity may affect acute toxicity estimates. Nonetheless, even in the individual studies which tested a variety of different species from different families, no clear pattern of species or family sensitivity emerges. Although Macek and McAllister (1970) were able to demonstrate substantial and consistent differences in the response of various families of fish to carbamate and organophosphorous insecticides, no such differences were observed with the organochlorine insecticides DDT, lindane, and toxaphene. Of

Table 5.10. Acute toxicity of toxaphene to various fish during static exposures

FAMILY Organism Common name (Genus species)	Size Average (range) wgt. in grams Length in mm	Temperature °C	Other conditions	LC$_{50}$ in ppb after specified interval (95% confidence interval)					Reference
				24 hr	36 hr	48 hr	72 hr	96 hr	
CENTRARCHIDAE									
Bluegill sunfish (Lepomis macrochirus)	2.9 g	20°	pH 7.4, hardness 24 ppm		23				Ferguson et al., 1964
	(1-2 g)	25°	pH 7.4, hardness 20 ppm	7.5		3.8		3.5	Henderson et al., 1959
	-	18°	pH 7.1, alk. 35 ppm					18(10-30)	Macek and McAllister, 1970
	(0.6 1.5 g)	18.3°	pH 7.1, alk. 35 ppm	6.8(6.2-7.5)				2.6(2.2-3.0)	Macek et al., 1969
Green sunfish (Lepomis cyanellus)	3.17 g	20°	pH 7.4, hardness 24 ppm		38				Ferguson et al., 1964
Redear sunfish (Lepomis microlophus)	-	18°	pH 7.1 alk. 35 ppm					13(8-17)	Macek and McAllister, 1970
Largemouth bass (Micropterus salmoides)	-	18°	pH 7.4 alk. 35 ppm					2(1-3)	Macek and McAllister, 1970
CYPRINIDAE									
Carp (Cyprinus carpio)	-	18°	pH 7.1, alk. 35 ppm			56(48-67)			Ludemann and Neumann, 1960
								4(3-5)	Macek and McAllister, 1970
Goldfish (Carassius auratus)	(1-2 g) (38-64 mm)	25°	pH 7.4	8.2		6.8		5.6	Henderson et al., 1959
	-	18°	pH 7.1, alk. 35 ppm					14(11-19)	Macek and McAllister, 1970
	-	17.2°	pH 7.9, alk. 36 ppm	86		35	28	28	Mahdi, 1966
Golden shiner (Notemigonus crysoleucas)	2.43 g	20°	pH 7.4, hardness 24 ppm		30				Ferguson et al., 1964
	-	17.2°	pH 7.9, alk. 36 ppm	27		7.0	6.2	< 5.0	Mahdi, 1966
minnow, Blantnose (Pimephales notatus)	-	17.2°	pH 7.9, alk. 36 ppm	200		35	14	8.8	Mahdi, 1966

Table 5.10 (continued)

FAMILY Organism Common name (Genus species)	Size Average (range) wgt. in grams Length in mm	Temperature °C	Other conditions	LC50 in ppb after specified interval (95% confidence interval) 24 hr	36 hr	48 hr	72 hr	96 hr	Reference
CYPRINIDAE (cont'd)									
minnow, Fathead	(1.0-1.5 g)(51-64 mm)	25°	-					13	Cohen et al., 1960
(Pimephales promelas)	(1.0-2.0 g)(38-64 mm)	25°	pH 7.4, hardness 20 ppm	13		7.5		7.5	Henderson et al., 1959
	(51-64 mm)	24°	pH 7.4, alk. 212 ppm	5.7(4.1-8.0)					Hooper and Grzenda, 1957
	-	18°	pH 7.1, alk. 35 ppm					14(9-22)	Macek and McAllister, 1970
Stoneroller (Campostoma anomalum)	-	17.2°	pH 7.9, alk. 36 ppm	54		44	35	32	Mahdi, 1966
GASTEROSTEIDAE									
Threespine stickleback	(0.38-0.77 g)(22-44 mm)	20°	pH 7.1, salinity 5%			12	9.8	8.6	Katz, 1961
(Gasterosteus aculeatus)	"	"	pH 7.1, salinity 25%				8.8	7.8	
ICTALURIDAE									
Black bullhead	-	20°	pH 7.4, hardness 24 ppm		3.75				Ferguson et al., 1965 a
(Ictalurus melas)	-	18°	pH 7.1, alk. 35 ppm					5(4-7)	Macek and McAllister, 1970
	-	17.2°	pH 7.9, alk. 36 ppm	15		4.3	4.2	2.7	Mahdi, 1966
Channel catfish (Ictalurus punctatus)	-	18°	pH 7.1, alk. 35 ppm					13(10-17)	Macek and McAllister, 1970
PERCIDAE									
Yellow perch (Perca flavescens)	-	18°	pH 7.1, alk. 35 ppm					12(9-14)	Macek and McAllister, 1970
POECILIIDAE									
Guppy (Lebistes reticulatus)	(0.1-0.2 g)(19-25 ppm)	25°	pH 7.4, hardness 20 ppm	42		24		20	Henderson et al., 1959

Table 5.10 (continued)

FAMILY Organism Common name (Genus species)	Size Average (range) wgt. in grams Length in mm	Temperature °C	Other conditions	LC_{50} in ppb after specified interval (95% confidence interval) 24 hr	36 hr	48 hr	72 hr	96 hr	Reference
POECILIIDAE (cont'd)									
Mosquito fish (Gambusia affinis)	-	21°	-		10				Boyd and Ferguson, 1964
	0.28 g	24°	pH 7.8, hardness 28 ppm		1.0				Burke and Ferguson, 1969
	(30-40 mm)	-	-	45		24	9	8	Chaiyarach et al., 1975
	-	-	-			31(LC_{90},63)			Dziuk and Plapp, 1973
	-	20°	pH 7.4, hardness 24 ppm		10				Ferguson et al., 1965 a
	1.08 g	-	-	860					Schaper and Crowder, 1976
SALMONIDAE									
salmon, Chinook (Oncorhynchus tshawytscha)	(1.45-5 g)(51-114 mm)	20°	pH 7.1, alk. 51 ppm	7.9		3.3	2.7	2.5	Katz, 1961
salmon, Coho (Oncorhynchus kisutch)	(2.7-4.1 g)(57-76 mm)	20°	pH 7.1, alk. 51 ppm	13.0		10.5	10.0	9.4	Katz, 1961
	-	13°	pH 7.1, alk. 35 ppm					8(6-10)	Macek and McAllister, 1970
trout, Brook (Salvelinus fontinalis)	16 months old	-	-					10.8	Mayer et al., 1975
trout, Brown (Salmo trutta)	-	13°	pH 7.1, alk. 35 ppm					3(2-5)	Macek and McAllister, 1970
trout, Rainbow (Salmo gairdneri)	1 g	12.8°	-	7.6		4.4		2.7	Cope, 1965
	3.2 g (51-79 mm)	20°	pH 7.1, alk. 51 ppm	11.5		8.4	8.4	8.4	Katz, 1961
	-	13°	pH 7.1, alk. 35 ppm					11(8-13)	Macek and McAllister, 1970
	-	12°	pH 7.9, alk. 36 ppm	22		14.5	11.1	8.4	Mahdi, 1966
SERRANIDAE									
Striped bass (Morone saxatilis)	2.3 g (60 mm)	17°	salinity 30%					4.4(2-9)	Korn and Earnest, 1974

the twelve species of fish tested by Macek and MacAllister (1970), bullheads (ictalurids), carp (cyprinids), largemouth bass (centrarchids), and brown trout (salmonids) were significantly more susceptible to toxaphene than the remaining eight species. The four susceptible species had 96-hour LC50 values ranging from 2 to 5 ppb, while the remaining species had a corresponding range of 8 to 14 ppb. In all but one instance, the LC50's for the sensitive species were significantly different (p less than 0.05) from those of the other species. Thus, while sensitive and tolerant species of fish were found, the pattern of sensitivity did not fall into family groups. The centrarchids contained both the most and least sensitive species: the largemouth bass with a 96-hour LC50 of 2 ppb and the bluegill sunfish with a 96-hour LC50 of 18 ppb.

An examination of the results of other studies summarized in Table 5.10 which used more than one species of fish also reveals no clear patterns of species or family sensitivity. Of the six species of fish tested by Mahdi (1966), the black bullhead (ictaluridae) was more susceptible to toxaphene than any of the four species of cyprinids at 17 C but not at 11.6 C. Further, at the lower temperature, the rainbow trout (salmonid) was more susceptible (LC50 8.4 ppb) than the ictalurid or any of the cyprinids (LC50's 14 to 94 ppb). Similarly, Katz (1961) found no remarkable differences in the toxicity of toxaphene to three species of salmonids and one gasterosteid. Given the marked effect that preexposure, genetic resistance, and a variety of experimental parameters can have on such acute toxicity estimates (following paragraphs and Section 5.2.2.2.4), the relatively narrow range of LC50's noted in Table 5.10 does not suggest major species or family specific differences in the acute toxicity of toxaphene to fish.

In the treatment of freshwater lakes with toxaphene as a piscicide, various investigators have noted that certain lakes remain toxic to fish for longer periods of time than other lakes receiving comparable doses of toxaphene (Section 5.2.2.2.5). While these findings could be attributable to differing rates of toxaphene breakdown in different aquatic systems (Section 7.3.2), the possibility that different toxaphene formulations or varying water quality characteristics might also affect the toxicity of toxaphene to fish also has been examined. Based on the current available information, detailed below, substantial (greater than 10 C) increases in temperature do seem to increase the toxicity of toxaphene to most fish, but the increase is generally less than a factor of five. Other water quality variables, such as pH, alkalinity, hardness, and salinity have less pronounced effects, and the formulation of toxaphene used seems to have little, if any, effect. None of these factors approach, in magnitude, an apparent importance of preexposure or genetic resistance (Section 5.2.2.2.4).

Most toxicants have a positive temperature coefficient - i.e., toxicity increases with increasing temperature. Macek and coworkers (1969) found this relationship to hold for a variety of commercial pesticides including toxaphene. As summarized in Table 5.11, most of the available data on the toxicity of toxaphene to fish also indicate a positive temperature coefficient. In all but one instance where temperature differs by more than 10 C, the toxicity of toxaphene is increased at the higher temperature. The single exception, the 24-hour LC50 values for the goldenshiner, is of questionable significance because confidence intervals are not given and because a positive temperature coefficient is suggested at 48 and 72 hours.

In general, the magnitude of the positive temperature coefficient seems to decrease or stay about the same as the exposure period increases. For the

Table 5.11. Effects of temperature in the acute toxicity of toxaphene to fish

FAMILY Organism	Temperature °C	LC_{50} in ppb (95% confidence interval) 24 hr	48 hr	72 hr	96 hr	R.I.T.[a]	Reference
CENTRARCHIDAE							
Bluegill sunfish	12.7	9.7 (8.4-11)			3.2 (2.8-3.7)	1.3	Macek et al., 1969
	18.3	6.8 (6.2-7.5)			2.6 (2.2-3.0)		
	23.8	6.6 (5.5-7.9)			2.4 (2.1-2.7)		
CYPRINIDAE							
Fathead minnow	10	20 (14-28)				3.5[b]	Hooper and Grzenda, 1957
	24	5.7 (4.1-8.0)					
Stoneroller	11.6	62	27	27	14	> 2.8	Mahdi, 1966
	17.2	54	44	35	32		
	22.8	9	7.8	< 5	< 5		
Goldfish	11.6	270	115	94	94	1.9	
	17.2	86	35	28	28		
	22.8	54	50	50	50		
Bluntnose minnow	11.6	> 4000	860	150	30	4.8	
	17.2	200	35	14	8.8		
	22.8	15	8.4	6.5	6.3		
Goldenshiner	11.6	12.5				0.93[b]	
	17.2	27	7	6.2	< 5		
	22.8	13.4	6.6	6.0	6		
ICTALURIDAE							
Black bullhead	11.6	> 48	> 48	34	25	13.9	Mahdi, 1966
	17.2	15	4.3	4.2	2.7		
	22.8	12	4.2	3	1.8		
SALMONIDAE							
Rainbow trout	7.2	16.0	8.4		5.4	3.0	Cope, 1965
	12.7	7.6	4.4		2.7		
	18.3	5.0	2.8		1.8		

[a] R.I.T. - Relative increase in toxicity: LC_{50} at highest temperature divided by LC_{50} at lowest temperature. 96 hr value used unless otherwise specified.

[b] 24 hr LC_{50}'s used.

bluntnose minnow, the R.I.T. (relative increase in toxicity as defined in Table 5.11) between 11.6 C and 22.8 C is more than 267 in 24-hour exposures but drops to 4.8 in 96-hour exposures. The only apparent exception to this pattern is the black bullhead which has R.I.T. values of greater than 4 and 13.9 at 24 hours and 96 hours, respectively. As with the estimates of acute toxicity discussed above, the rather wide variability found within the cyprinids, and the limited information available on the other families, cautions against general statements on the family or species sensitivity to the temperature effect. However, it is interesting to note that the black bullhead, which seems to be among the fish more sensitive to toxaphene, also has a markedly higher R.I.T. than the other species listed in Table 5.11.

In addition to temperature, a variety of other water characteristics such as pH, alkalinity, hardness, and salinity, could affect measurements of acute toxicity. However, relatively little detailed information is available in these factors in estimates of toxaphene toxicity. Hooper and Grzenda (1957) noted a slight, but not statistically significant, increase in the toxicity of toxaphene to fathead minnows over 24-hour exposures at 10 C with increasing alkalinity. At an alkalinity of 6 ppm, the LC50 (95 percent confidence interval) was 36 (26 to 50) ppb. When the alkalinity was raised to 212 ppm, the corresponding toxicity estimate was 20 (14 to 28) ppb. Using the same species, Henderson and coworkers (1959) also noted a slight increase in the toxicity of toxaphene at alkalinities of 18 ppm and 360 ppm with corresponding LC50 values of 7.5 ppb and 5.1 ppb.

The effect of pH has not been systematically evaluated. Workman and Neuhold (1963) tested goldfish and mosquito fish using two toxaphene formulations in waters from three different lakes with pH values of 7.0, 7.8, and 8.3.

Statistically significant differences among the various waters were uncommon. In that the lakes also differed markedly in dissolved solids (46 to 238 ppm), the significant differences which were found could not be unequivocally attributed to pH.

The only other water quality characteristic which has received any attention is salinity. As summarized in Table 5.10, Katz (1961) demonstrated a slight increase in the toxicity of toxaphene to the threespine stickleback (Gasterosteidae) as salinity increased from 5 parts per thousand to 25 parts per thousand.

No information is available on the effect of dissolved oxygen on toxaphene fish toxicity. Courtenay and Roberts (1973) attempted to obtain such information but were unsuccessful due to problems in exposure technique. This is particularly regrettable, given the speculations of Schaper and Crowder (1976) described below, that respiratory distress may be a major factor in acute toxaphene exposures.

Toxaphene is or has been available in a variety of formulations. The studies summarized in Table 5.10 identify the toxaphene used as commercial formulations with toxaphene concentrations of 0.48 kg/liter, 0.72 kg/liter, or 0.96 kg/liter, or as "pure" or technical grade toxaphene. Those studies which used pure or technical toxaphene in a specified solvent (usually acetone) conducted appropriate solvent controls, the details of which are not included in this report. No systematic studies are available on the potential effect of the various solvents or emulsifying agents used in commercial formulations on the toxicity of toxaphene to fish. Two studies, however, have compared the potency of different toxaphene formulations. Mayhew (1955) exposed fingerling rainbow trout to toxaphene as an emulsifiable concentrate or wettable powder at concentrations (as toxaphene) ranging from 50 to 1000 ppb. No fish survived exposure to the lowest concentration of either formulation for over 16 hours. Because of this high mortality, standard LC50 values cannot be calculated. Nevertheless, the emulsifiable concentrate seemed slightly more toxic, killing all fish exposed to 100 ppb after 12 hours. Identical exposure to the wettable powder killed 70 percent of the fish. Similarly, Workman and Neuhold (1963) compared the toxicity of sinking and floating formulations of toxaphene. Using various combinations of temperature, water type, and species, statistically significant differences between these two formulations were demonstrated in only one of eight trials. Thus, there is no clear indication that the formulation of toxaphene used markedly affects the toxicity estimates given in Table 5.10.

The mechanism by which toxaphene adversely affects fish is not known. Like most chlorinated hydrocarbon insecticides, toxaphene is assumed to act primarily in the nervous system, and such an assumption is consistent with most of the signs of toxaphene poisoning in fish. Henderson and coworkers (1959) found that the responses of fathead minnows, bluegills, goldfish, and guppies to nine chlorinated hydrocarbon pesticides, including toxaphene, were essentially the same. Initially, these fish displayed hyperactivity followed by periods of muscular spasms. As intoxication progressed, the major sign of poisoning was loss of equilibrium accompanied by periodic short, jerky movements. Similar patterns have been seen in carp, tilapia, trout, bullheads, mosquito fish, and goldfish (Gruber, 1959; Kallman et al., 1962; Ludemann and Neumann, 1960; Schaper and Crowder, 1976; Workman and Neuhold, 1963). The period of initial hyperactivity may be concentration dependent. Workman and Neuhold (1963) note this effect only at water concentrations of more than 3 ppm toxaphene. The loss of equilibrium seen in the latter stages of intoxication is characterized by partial paralysis, impaired locomotion, and a loss of dorso-ventral orientation with the fish turning on to their sides either at the surface of the water or the bottom of the exposure vessel (Ludemann and Neumann, 1960; Workmann and Neuhold, 1963). In addition to these signs of apparent nervous system damage, Schaper and Crowder (1976) have noted signs of respiratory distress in mosquito fish. Shortly after exposure to 2 ppm toxaphene, fish began to display swimming behavior characteristic of low water oxygen levels, and rapid gill ventilation was noted throughout the exposure. As discussed in Section 5.3, suffocation rather than nerve damage has been proposed as a mechanism of toxaphene's effect on aquatic invertebrates.

Little detailed information is available on the total body burden of toxaphene necessary to cause signs of intoxication in fish. Bullheads showing advance signs of toxaphene poisoning after three to six days exposure in a toxaphene-treated lake had total body toxaphene concentrations of 8 to 15 ppm (Kallman et al., 1962). However, in mosquito fish showing similar signs of intoxication after exposure to 2 ppm toxaphene for up to eight hours, total body residues of only 0.42 to 0.75 ppm were found (Schaper and Crowder, 1976).

Compared to other common pesticides, toxaphene is an extremely potent fish toxicant on acute exposure. Table 5.12 summarizes some of the available information on the toxicity of toxaphene relative to some other pesticides. Information on additional pesticides and other fish species is given in the studies cited in this table. With the exception of endrin, toxaphene is almost invariably more toxic to fish than other chlorinated hydrocarbons and consistently more toxic than organophosphorous or carbamate pesticides.

Table 5.12. The relative acute toxicity to fish of some common pesticides compared to toxaphene[a]

	Fish				
Pesticides	Carp[b]	Rainbow trout[c]	Bluegills[d]	Striped bass[e]	Bullhead[f]
Endrin	14.0	8.0	5.8	47.0	-
Dieldrin	0.83	0.80	0.44	0.22	-
DDT	0.98	0.80	0.22	8.3	1.0
Heptachlor	0.15	0.44	0.18	1.5	-
Lindane	0.20	0.18	0.045	0.60	0.078
Malathion	0.003	0.005		0.31	0.00039
Parathion	0.016	-	-	0.25	-
Carbaryl	-	-	-	0.0044	0.00025

[a] Relative toxicity calculated as LC_{50} of toxaphene $\div$ LC_{50} of specified pesticide.
[b] Source: Ludemann and Neumann, 1960.
[c] Source: Cope, 1966.
[d] Source: Henderson et al., 1959.
[e] Source: Korn and Earnest, 1974.
[f] Source: Macek and McAllister, 1970.

Other topics dealing with the acute toxicity of toxaphene to fish are resistance/tolerance and drug interactions, which are discussed in Sections 5.2.2.2.4 and 5.3.2.2.7, respectively.

The fish toxicity of individual toxaphene components has received relatively little attention. A mixture of two toxaphene components, 2,2,5-endo,6-exo,8,8,9,10-octachloroborane and 2,5,5-endo,6-exo,8,9,9,10-octachloroborane (toxicant A), is about twenty-five times more toxic to goldfish than technical toxaphene and has a 24-hour LC50 of 1.7 ppb to this species. Another component, 2,2,5-endo, 6-exo,8,9,10-heptachloroborane (toxicant B) is only about five times more toxic to this species than technical toxaphene, having a 24-hour LC50 of 8.6 ppb (Turner et al., 1975). Similar patterns have been noted in the toxicity of these components to mice (Table 6.13, Section 6.2.2.1.2) and houseflies (Table 5.61, Section 5.6.2.2.1.2).

5.2.2.2.2 Subacute and chronic toxicity -- Relatively few controlled studies are available on the effects of prolonged exposures of fish to toxaphene. While studies on fish kill in toxaphene-treated lakes can be used to supplement the available experimental information, such studies are inherently epidemiological, involving ill-defined dosage data, uncontrolled, and often undefined exposure conditions, and few details on toxic response other than mortality estimates. Consequently, such case studies are discussed separately (Section 5.2.2.2.5).

As described in Section 5.2.2.1, Mayer and coworkers (Mayer and Mehrle, 1976; Mayer et al., 1975; Merhle and Mayer, 1975 a, b) have demonstrated that prolonged toxaphene exposure causes decreased spinal collagen in fathead minnows, brook trout, and channel catfish. Using fathead minnows, Mehrle and Mayer (1975 a) related this biochemical effect directly to increased backbone fragility. Six fish from each toxaphene-treated group (0.055 ppb to 0.621 ppb for

150 days) were exposed to sublethal electrical shocks. In each instance, four of the six fish had multiple complete fractures of the spine. No such breaks were seen in control fish given identical electrical shock treatments. In addition to spinal weakness, all exposures resulted in decreased growth by 150 days (Table 5.13). However, although such growth depression was statistically significant, the extent of depression ranged from only 4 percent to 22 percent. As with the effects on collagen synthesis, the extent of growth retardation was not dose-related. All of these chronic effects were associated with total body residues of 5.9 to 52 ppm. Excessive mortality was seen only in fish exposed to 1.23 ppb for 150 days (Mehrle and Mayer, 1975 a).

Although these investigators did not attempt to demonstrate spinal fragility in other fish species, comparable studies on growth and mortality in brook trout suggest that this game fish is markedly more sensitive to toxaphene than fathead minnows. Brook trout fry exposed to toxaphene at concentrations as low as 0.039 ppb evidenced significantly decreased growth after 60 days (Table 5.14). With these fish, the extent of growth depression was approximately 50 percent. This effect was associated with total body residues generally under 1 ppb. Thus, the increased sensitivity of these fish compared to minnows is not attributable to more rapid or complete absorption. Further, complete mortality was seen in all fry exposed to 0.288 ppb for 60 days and 0.502 ppb for 30 days (Mehrle and Mayer, 1975 b). Yearling brook trout are somewhat less susceptible than fry (Mayer et al., 1975). At exposures of 0.270 ppb for 180 days, growth of yearlings, expressed both as length and weight gain, was depressed 24 percent. In similar exposures to 0.5 ppb, length was reduced 24 percent and weight gain reduced 46 percent. All of these differences from control fish were significant at p less than 0.05. In addition to growth

Table 5.13. The effects of chronic toxaphene exposure on the growth of fathead minnows[a]

Days of exposure	Measurement	Toxaphene concentration (ppb)					F value[b]
		0	0.055	0.132	0.288	0.621	
60	length (mm)	30.4	30.2	30.6	30.1	30.0	0.34
	weight (g)	0.27	0.27	0.28	0.27	0.27	0.42
90	length (mm)	40.0	39.2	39.2	38.9	38.8	0.76
	weight (g)	0.68	0.66	0.64	0.63	0.62	0.52
150	length (mm)	48.6	46.2[c]	45.2[c]	46.6[c]	46.2[c]	2.44
	weight (g)	1.28	1.14[c]	1.01[c]	1.14[c]	1.04[c]	2.52

[a] Source: Mehrle and Mayer, 1975 a.

[b] $F_{.05} = 2.26$; $F_{.01} = 3.12$.

[c] Significantly different from controls ($LSD_{0.05}$).

Table 5.14. The effect of chronic toxaphene exposure in the growth of brook trout fry[a]

Toxaphene concn (ppb)	Fry length in mm (standard deviation in parentheses)				Weight (g) at 90 days
	0 day	30 days	60 days	90 days	
0	16.5 (0.8)	23.4 (1.2)	34.3 (4.4)	42.0 (9.2)	0.81 (0.60)
0.039	16.8 (1.1)	23.3 (2.2)	31.4[b] (3.3)	35.8[b] (6.2)	0.44[b] (0.30)
0.068	16.8 (1.1)	23.6 (2.0)	31.3[b] (3.4)	38.3[b] (8.2)	0.59[b] 0.44
0.139	16.8 (1.2)	22.0[b] (2.1)	30.8[b] (4.3)	35.1[b] (7.7)	0.43[b] (0.37)
0.288	16.3 (1.0)	19.4[b] (1.6)	–[c]	–[c]	–[c]
0.502	16.4 (1.0)	–[c]	–[c]	–[c]	–[c]

[a] Source: Mehrle and Mayer, 1975 b.

[b] Significantly different from controls ($LSD_{0.05}$).

[c] All fish dead.

depression, hyperexcitability, discoloration, and increased incidences of fin erosion were seen in these fish. The subacute toxicity of toxaphene to yearling trout, expressed as 14 day LC50 (95 percent confidence interval), was 3.6 (3.2 to 4.0) ppb. Although mortality data from the chronic exposure was not suitable for calculating a chronic LC50, Mayer and coworkers (1975) indicate that 180 day exposures to 0.288 ppb caused about 50 percent mortality in yearling brook trout.

Various pathological lesions have been reported in fish after subacute or chronic exposure to toxaphene. Like many chlorinated hydrocarbon pesticides, toxaphene seems to cause histological alteration of liver tissue. In five-month exposures of *Leiostomus xanthurus* (spot) to toxaphene concentrations of 0.01 ppb and 0.1 ppb, no growth retardation was noted, but unspecified degenerative changes were seen in the livers of treated fish (Lowe, 1964). Similarly, Cope (1966) also reports liver damage in the trout exposed to toxaphene (details of exposure not specified). In toxaphene exposures of from 1 ppb to 100 ppb, carp displayed dose-related ultrastructural changes in hepatic cells characteristic of enzyme induction. Such changes included proliferation of the rough endoplasmic reticulum, swelling of the mitochondria, and condensation of nuclear chromatin (Rutschke and Brozio, 1975).

In addition to liver degeneration, Lowe (1964) reports a pronounced thickening in the gill lamellae of toxaphene-treated fish but no damage to renal or nervous tissue. However, in the fry of largemouth bass given toxaphene in food at 10 percent of the 96-hour TLm for 14 days, Courtenay and Roberts (1973) found extensive necrosis of kidney tissue with almost complete degeneration of the kidney tubules. Necrotic changes were also seen in the lining of the digestive tract. The only positive report of nervous system damage is given by Kayser and coworkers (1962). In this instance, cell swelling, chromatolysis, and agglutination of the Nissal substance around the cell nuclei were found in the brain cells of carp intoxicated with toxaphene.

5.2.2.2.3 Reproductive and teratogenic effects -- Toxaphene seems to have an adverse effect on egg development when exposure occurs via gravid females. Boyd (1964) noted that 5 percent of the gravid mosquito fish aborted after surviving toxaphene exposures that caused 10 to 40 percent mortality (toxaphene concentration not specified). Similarly, egg viability was markedly reduced when spawning female brook trout were exposed to toxaphene concentrations of 0.075 ppb and above (Table 5.15). However, eggs obtained from females not exposed to toxaphene were incubated in solutions of 0.039 ppb to 0.502 ppb toxaphene for twenty days prior to hatching and no decrease in egg hatchability was seen (Mayer et al., 1975; Mehrle and Mayer, 1975 b).

Courtenay and Roberts (1973) have noted developmental abnormalities in striped mullit (*Mugil capbalus*) embryos exposed to toxaphene concentrations of up to 0.5 ppm (more detailed dosage data not available). These organisms exhibited enlargement and proliferation of glandular structures, apparently mucous glands, in the optic region. Because such changes might be attributable to epithelial irritation rather than to actual maldevelopment, the teratogenic implications of these findings are questionable (Courtenay and Roberts, 1973).

5.2.2.2.4 Resistance/tolerance -- Ferguson and coworkers (Boyd and Ferguson, 1964; Burke and Ferguson, 1969; Ferguson et al., 1964, 1965 a) were the first to note that fish of the same species taken from different locations differed substantially in their susceptibility to toxaphene. Boyd and Ferguson (1964) associated these differences with different levels of preexposure of fish populations to pesticide runoffs. Mosquito fish were collected from four areas of Mississippi, one of which was reasonably free of pesticide contamination, and

Table 5.15. The effect of toxaphene on brook trout reproduction[a]

Exposure, ppb	Number of females spawning	Number of spawns	Eggs per spawn	Eggs per female	% Viability
Control[b]	4	9	380	855	69
0.041	6	8	406	541	60
0.075	6	9	344	516	22[c]
0.125	6	12	271	542	15[c]
0.270	4	7	264	462	6[c]
0.500	1	1	617	617	0[c]

[a] Source: Mayer et al., 1975.
[b] Nominal concentration.
[c] Significantly different from controls (P less than 0.05).

allowed to breed in control ponds containing no pesticides. The fish population from the area free of pesticides, which was designated as susceptible, had a 36-hour LC50 to toxaphene of 10 ppb. Fish populations from the other areas had LC50's of 60 ppb to 480 ppb. In that these LC50's were obtained from second or third generation offspring, genetic resistance rather than some form of preexposure tolerance was proposed. In a subsequent study on mosquito fish, Burke and Ferguson (1969) identified a fish population 200 times more resistant to toxaphene than the susceptible population. However, dose response curves for both populations of fish had about the same slope (Figure 5.4). Similar patterns of resistance have been reported in mosquito fish populations in Texas (Dziuk and Plapp, 1973). Subsequent studies also found evidence of resistance in other species of fish. As summarized in Table 5.10, Ferguson and coworkers (1964) determined 36-hour LC50's for susceptible strains of goldenshiners, bluegills, and green sunfish at 30 ppb, 38 ppb, and 62 ppb, respectively. However, corresponding LC50's in the same three species of fish collected in areas of high pesticide runoff were 1200 ppb, 1600 ppb, and 1500 ppb, respectively, giving resistance factors of from 39 to 70. In a similar study, various populations of black bullheads were found to differ from each other in their susceptibility to toxaphene by a factor of up to 13. In general, all of these studies indicated that resistance was developed not only to toxaphene but also to a variety of other chlorinated pesticides.

Further supporting the conclusion of Boyd and Ferguson (1964) that fish populations in highly contaminated areas possess genetic resistance rather than some form of tolerance, Lowe (1964) found that preexposure of spots to toxaphene concentrations of 0.01 ppb or 0.1 ppb for five months did not increase the tolerance of these fish to acute toxaphene poisoning. In fact, fish chronically

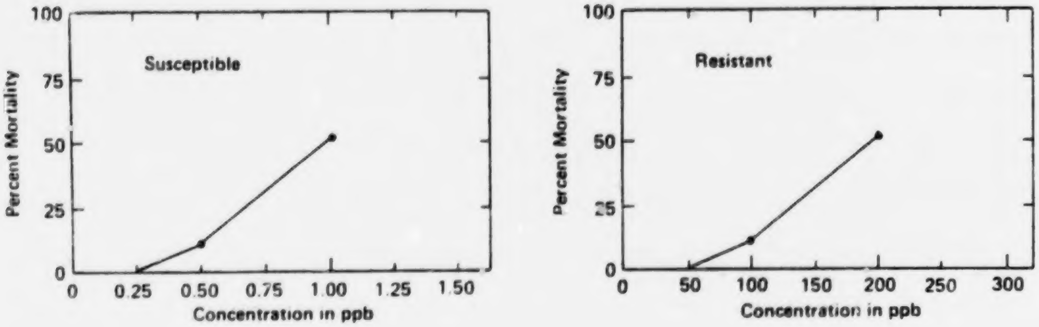


Figure 5.4. Dose-response curves for susceptible and resistant strains of mosquito fish exposed to toxaphene for 36 hours. Source: Burke and Ferguson, 1969.

exposed to sublethal levels of toxaphene were more sensitive to acute doses than fish with no history of preexposure. While it thus seems probable that fish populations in areas contaminated with pesticides do possess genetic resistance to toxaphene poisoning, the mechanisms of such resistance are not known. Finley and coworkers (1970) have suggested that high levels of pesticides, particularly DDT and toxaphene which appear in freshwater after heavy rains, may exert the primary selection pressure in the development of resistance. Fabacher and Chambers (1971) have noted some correlation between resistance and increased body lipids in mosquito fish. However, patterns of cross-resistance made it unlikely that this factor alone could account for resistant populations. In studies on the inhibition of succinic dehydrogenase in mitochondrial preparations from resistant and susceptible mosquito fish, Moffett and Yarbrough (1972) noted that the patterns of inhibition between mitochondria with intact and disrupted membranes suggested a relationship between resistance and membrane permeability. However, no relationship was found between acute toxicity data and the patterns of the in vitro enzyme inhibition.

Whatever the mechanism of genetic resistance, such resistance can alter the toxicity of toxaphene to a given species of fish by a factor of up to several hundred. Recalling the mere 16-fold difference in the 96-hour LC50 estimates for the various species of fish covered in Table 5.10, it seems apparent that preexposure conditioning, rather than the phylogenetic position of a given fish species, will largely determine the ability of a fish population in the environment to tolerate acutely toxic doses of toxaphene.

5.2.2.2.5 Toxaphene field studies -- Toxaphene once enjoyed wide usage as a piscicide in rough fish control programs. Such programs usually involved complete elimination of fish in a given lake. After a suitable period of detoxication, the lake was then restocked with the desired species of game fish. Such programs, which have been conducted since 1948, have been exhaustively reviewed by Hughes (1970). In terms of assessing the potential adverse effects of toxaphene in the environment, such studies must be interpreted with caution. Perhaps the greatest problem is determining the precise extent of fish exposure to toxaphene. Table 5.16, which summarizes several studies on toxaphene use as a fish poison, expresses toxaphene exposure as a theoretical dose. This is defined as the concentration of toxaphene which would be present in the water if the total amount of toxaphene added to the water mixed evenly and remained stable. This does not happen during the actual application of toxaphene. Kallman and coworkers (1962) found radical differences in the concentration of toxaphene in treated lake water for periods of up to 73 days after initial application. Toxaphene concentrations were usually higher on the leeward side of the lake and also appeared to be greater in deeper water. Further, toxaphene concentration never reached the theoretical maximum of 50 ppb, remaining below 10 ppb for most measurements. Similar failures to reach the theoretical dose have been noted by Hughes and Lee (1973), who partially attributed this to the water insolubility of toxaphene. Additional factors which might cause the rapid elimination of toxaphene from lake water are discussed in Section 7.3.2.

While these studies cannot be used to quantitatively refine estimates of toxaphene fish toxicity, they are eminently useful in indicating what does not happen under actual conditions of environmental exposure. As described in Section 5.2.2.2.3, toxaphene has been shown to have a marked effect on egg viability in fish at a concentration of 0.075 ppb. This information would certainly seem to suggest that toxaphene, at very low levels, would profoundly

Table 5.16. Use of toxaphene as a fish control agent

Location	Number of lakes treated	Characteristics of the lakes	Theoretical concentration	Fish mortality data	Detoxication data	Reference
North Dakota	16	2.6-370 ha, maximum depth of 2.4-8 meters pH 3.2-9.9, hardness 51-2,385	5 - 35 ppb	Incomplete to complete fish kills Smaller fish more susceptible than larger fish Minimum dose for complete kill, 20 ppb No correlations of toxicity to water chemistry	5 of 7 lakes detoxified after 7 months No data available on other lakes	Henegar, 1966 and Warnick, 1966
Wisconsin	4	5.3 - 1063 ha, maximum depth 5-9.8 meters pH 8.2-8.6, alk. 104-128 ppm	100 - 420 ppb	Complete kills of bullhead and stunted bluegill populations	Successful restocking after 7 - 10 months	Hughes and Lee, 1973 and Hughes, 1970
Wisconsin	8	24-110 ha, depth 3.5-8 meters pH 8.0-9.2	100 ppb	-	Toxaphene in water at 1 - 4 ppb for up to nine years after treatment	Johnson et al., 1966
New Mexico	1	32 ha, depth 5.7 meters pH 8.5, alkalinity 114 ppm	50 ppb	Substantial fish kill, 112 kg/ha	Successful restocking after 12 months Toxaphene in water at less than 1 ppb by 523 days after treatment	Kallman et al., 1962
British Columbia	7	4-55 ha, depth 3.3-16 meters pH 7.9-8.4	10 - 100 ppb	Complete fish kills	Lakes still toxic to fish after 9 months	Stringer and McMynn, 1958
Oregon	2	229 ha, 44 meters deep 1299 ha, 6 meters deep	40 ppb 88 ppb	Complete fish kills	Remained toxic for 6 years Remained toxic for 1 year	Terriere et al., 1966
Montana	1	5.2 ha, depth 4 meters pH 7.4-8.6	78 ppb	Complete fish kills Smaller fish more susceptible than larger fish	Remained toxic for 4 months	Wollitz, 1963

limit the development of stable fish populations in the environment. Based on the studies summarized in Table 5.16, such effects are not seen in toxaphene-treated lakes. After varying periods, most of these studies specify that the treated lakes were detoxified and were able to support productive and healthy fish populations. Both Hughes and Lee (1973) and Kallman and coworkers (1962) indicated that this happened in water containing 1 ppb toxaphene. The failure of toxaphene to affect fish productivity under such circumstances could be attributable to many factors. The most obvious of these is that the composition of toxaphene monitored in water after prolonged exposure periods is different from that of the originally applied commercial toxaphene. Hughes (1970) has reported that toxaphene extracted from treated lake water is less toxic to fish than commercial toxaphene. Thus, based on these fish control studies and other information given in Section 7.3.2, it may be reasonable to conclude that the toxic components in toxaphene that are responsible for the adverse effects on fish reproduction may be selectively removed from aquatic systems.

5.2.2.2.6 Behavioral effects -- Subtle effects on the behavioral patterns - e.g., feeding activity or predator avoidance - could substantially affect the ability of an organism to survive under normal environmental circumstances. Such affects, however, are difficult to document under controlled conditions. As previously discussed (Sections 5.2.2.2.1 and 5.2.2.2.2), toxaphene seems to cause hyperactivity in fish on both acute and chronic exposure. Using a highly complex experimental design, Warner and coworkers (1966) attempted to define less obvious behavioral changes. In this experiment, goldfish were continuously exposed to toxaphene at concentrations of 0.44 ppb and 1.8 ppb for 96 hours. The fish were kept in an exposure chamber designed both to detect and record the response of these fish to various external stimuli - e.g., light and shock. Comparing the behavior of exposed fish to controls, these investigators concluded that toxaphene causes "behavioral pathology of considerable severity." The nature of this pathology and the possible effects that it might have on goldfish under normal environmental stress are not described.

Kynard (1974) has demonstrated that both susceptible and resistant mosquito fish avoid toxaphene concentrations of 250 ppb and above.

5.2.2.2.7 Drug interactions -- There is no evidence that toxaphene has a synergistic or antagonistic effect with other pesticides in its effect on fish. Macek (1975) found that toxaphene/parathion and toxaphene/DDT combinations had only additive combined action in their acute toxicity to bluegills. Similar results have been reported for toxaphene/methyl parathion, toxaphene/DDT, and toxaphene/endrin combinations using mosquito fish (Ferguson and Bingham, 1966).

5.2.2.2.8 Effects on amphibians -- Very little attention is given to this group of animals by aquatic toxicologists, and this lack of concern is evidenced in the toxaphene literature. Nonetheless, based on the limited available data, these animals seem markedly less susceptible than fish to toxaphene. Sanders (1970) determined the acute toxicity of toxaphene to western chorus frog tadpoles (*Pseudacris triseriatu*) and one week old Fowler's toads (*Bufo woodhousii*). For the toad, 24, 48 and 96-hour LC50's with corresponding 95 percent confidence intervals were 600 (300 to 1200) ppb, 290 (200 to 420) ppb, and 140 (60 to 350) ppb, respectively. Even higher values were found with the tadpole; 1700 (500 to 3200) ppb, 700 (400 to 1200) ppb, and 500 (100 to 1100) ppb. The symptoms of toxaphene poisoning, however, were similar to those seen in fish and included initial excitability, loss of equilibrium, and death. Also similar to fish, toxaphene was among the more toxic pesticides to the toad, being exceeded only by endrin and trifluralin. Mulla (1962, 1963) recommended the use of toxaphene for frog and toad control. Although giving no LC50 estimate, Mulla (1963) indicated that toxaphene causes complete eradication of three species of frogs when applied at levels of about 1.2 kg/ha.

As with fish, Ferguson and Gilbert (1967) noted resistant or tolerant populations of frogs and toads, with the pattern of resistance/tolerance apparently related to preexposure to pesticide runoffs. In these animals, 36-hour LC50 values were extraordinarily high, ranging from 50 ppm to 500 ppm. No attempt was made to demonstrate a genetic basis to this lack of susceptibility. However, potential genetic resistance to DDT has been described in frogs (Boyd et al., 1963) and, given the pattern seen in fish, could also apply to toxaphene.

5.3 AQUATIC INVERTEBRATES

5.3.1 Metabolism

Information on the absorption, transport, distribution, biotransformation, and elimination of toxaphene in marine and freshwater invertebrates is not as detailed as in vertebrate species (Section 6.1). Butler et al. (1962) have demonstrated a failure of molluscs, oysters in particular, to continue the normal deposition of their shell when exposed continuously to 0.1 ppm, with differences detectable as early as 24 hours.

Metcalf (1955) suggests that, unlike cyclodiene insecticides, toxaphene is distributed throughout the invertebrate animal's body by the hemolymph and has no effect on the nervous system. Wagner (1974) states that in those aquatic organisms which are especially sensitive to this class of compounds, the uptake of oxygen through the gills is disrupted, and death is associated with suffocation rather than nervous disorders. In the marine environment the dehydrochlorination of this chemical under alkaline conditions may enhance its toxicity.

To date no studies in aquatic invertebrates have been found that utilized Cl-36 or C-14 labelled toxaphene. Any conclusions about sites of absorption, transport, and distribution within the organism and biotransformation of the compound are hard to predict without such studies. Similarities in many metabolic pathways and fate of the toxaphene may be common in many organisms and similar to those described in Section 6.1. However, unique species-specific metabolic differences are likely to exist.

Oysters exposed to 1 ppb toxaphene for 36 weeks bioaccumulated the compound throughout the exposure period until spawning, at which time there was a noticeable drop in tissue levels of toxaphene. Likewise, when oysters were exposed to a combination of DDT, toxaphene, and parathion, at 1 ppb each, there was bioaccumulation throughout the exposure period accompanied by the drop during spawning. Between four and twelve weeks after the removal of these oysters from the toxicants, they had eliminated the toxaphene from their tissues (Lowe et al., 1971). Table 5.17 characterizes these data on tissue accumulation. The drop in toxaphene levels following spawning indirectly suggests that the oyster can rid itself of a significant portion of its body load of toxicants in this manner.

Schoettger and Olive (1961) have reported that *Daphnia magna* and *D. pulex* accumulate sub-lethal doses of toxaphene. When these cladocerans serve as the food source for the kokanee salmon, *Orcorhynchus nerka kennerlyi*, and shiners, *Notropis sp.*, mortality is high in the fish (Table 5.18). This study suggests that toxaphene is stored unchanged or bound in the *Daphnia* in a form which remains toxic to other members of the food chain. It was not reported by Schoettger and Olive (1961) whether these *Daphnia* irreversibly bound the toxaphene, or if it might be lost when the organisms were returned to a toxaphene-free environment. The levels that are stated by Schoettger and Olive (1961) as sublethal to *Daphnia* and the accumulated dosages imply that the *Daphnia* and water in which they are living have equivalent levels of toxaphene. If they are in equilibrium with the toxaphene as suggested, then their introduction into the fish aquaria would be expected to leech out the toxaphene stored by the *Daphnia*. The mortality in the fish could then be the result of an interaction of toxaphene levels present in their food and in the aquaria water. Results of these experiments indicate that single sublethal dosages of toxaphene are inadequate to produce accumulations in fish-food organisms which cause mortalities among the test fish (Table 5.18). Multiple sublethal doses permitted sufficient amounts

Table 5.17. Accumulation of toxaphene by oysters exposed to 1 ppb singly or in combination with 1 ppb DDT and 1 ppb parathion[a]

Week	Pesticide residue (ppm of wet tissue weight)	
	Single exposure	Combination exposure
12	20.0	1.0
24	23.0	30.0
36[b]	8.0	9.0
40	-	3.0
44	-	ND[c]
48	-	ND

[a] Source: Modified from Lowe et al., 1971.

[b] End of pesticide exposure.

[c] Not detectable, less than 0.010 ppm.

Table 5.18. Effects of toxaphene exposed Daphnia on fish[a]

Trial no.	Dosage (pm)	Exposure period (hr)	Species of fish	No. of fish	Test fish no. of fish surviving after (hr) 24	48	72	96	120	144	Control fish no. of fish surviving after (hr) 96	120	144
1	0.03	36	O. nerka kennerlyi	4	4	4	4	4	4	-	4	4	-
2	0.03	72	O. nerka kennerlyi	4	4	4	4	4	4	-	4	4	-
3	0.03	96	O. nerka kennerlyi	4	4	4	4	4	-	-	4	-	-
4	0.03	192	O. nerka kennerlyi	4	4	4	4	4	4	4	4	4	4
5	0.03	216	O. nerka kennerlyi	8	7	7	7	6	6	5	8	8	7
6	0.03	288	O. nerka kennerlyi	4	4	4	4	4	4	4	4	4	4
7	0.03	312	O. nerka kennerlyi	8	8	8	8	8	-	-	8	-	-
8	0.03	408	O. nerka kennerlyi	4	4	4	4	4	4	4	4	4	4
9	0.03	624	O. nerka kennerlyi	4	4	4	4	4	4	4	4	4	4
10	0.01 0.13[b]	312	O. nerka kennerlyi	8	0	0	0	0	0	-	8	8	-
11	0.01 0.1	240	Notropis sp.	12	9	4	3	2	2	-	12	12	-
12	0.02 0.1	120	Notropis sp.	12	10	7	5	4	3		12	11	-

[a] Source: Modified from Schoettger and Olive, 1961.

[b] Total dosage of multiple, sublethal dosages.

of toxaphene to accumulate in Daphnia to cause a significant increase in mortality in kokanee salmon and in shiners. It might be concluded that sublethal, acute dosages are detoxified before they accumulate. However, sublethal, chronic dosages may exceed the detoxification rate and permit toxaphene to accumulate in the Daphnia.

Toxaphene residues were obtained from plankton in Big Bear Lake, California, following two toxaphene treatments of 0.03 and 0.10 ppm two weeks apart (Table 5.19). Bioaccumulation and biomagnification of toxaphene occurred for 114 days post-application. Values present in the plankton at this time were sometimes greater than the original application. At 265 days the values in the plankton were still greater than 1.5 times the original application (Johnson, 1966). The presence of this level of toxaphene in the plankton was toxic to the fish, and produced toxaphene levels in the fish that were considered unsafe for ten months post-application.

Naqvi (1973) states that tubificid worms (B. sowerbyi) are capable of accumulating toxaphene in large quantities in nature, and that worms collected in the Mississippi River Delta contained large amounts which he was unable to quantitate.

Bioconcentration in oysters (Lowe et al., 1971) and in plankton (Johnson, 1966) are documented. Bioconcentration is indicated in Daphnia (Schoettger and Olive, 1961). After the pesticide has entered the organism, it may be stored as residues, either unchanged for long periods or decomposition may take place in the animal body, with storage or excretion of the breakdown products. The fate of the pesticide and the capacity of aquatic animals to remove, store, or metabolize it may be related to the previous pesticide burden (Section 5.3.2.3), and the potential for food chain amplification at higher trophic levels exists.

Table 5.19. Toxaphene residue in plankton from Big Bear Lake exposed to 0.03 and 0.10 ppm treatments two weeks apart[a]

Date	Toxaphene residue (ppm)	Days since last treatment
Oct. 4, 1960	1.4	5
Oct. 19, 1960	50.0	21
Jan. 20, 1961	73.0	114
Jan. 20, 1961	97.0	114
Mar. 7, 1961	50.0	160
Apr. 10, 1961	20.0	194
Apr. 10, 1961	30.0	194
Apr. 20, 1961	16.0	204
May 11, 1961	17.0	225
June 13, 1961	19.0	251
June 20, 1961	10.0	258
June 20, 1961	12.0	258
June 26, 1961	12.0	264
June 26, 1961	0.0	264
June 26, 1961	19.0	264
June 26, 1961	trace	264
June 27, 1961	2.0	265
June 27, 1961	2.0	265
June 27, 1961	2.0	265

[a] Source: Modified from Johnson, 1966.

5.3.2 Effects

5.3.2.1 Physiological or Biochemical -- With expanding use of chemical pesticides it has become vital to understand their activity and eventual fate in the natural environment. By and large, we are aware of the initial animals or plants that these chemicals control or kill. However, our knowledge of their secondary effects is limited. It is probable that many of these substances and, possibly, their more toxic oxidation products, find their way into our watersheds, estuaries, and coastal waters. The coastal waters are important to us commercially as a home for many of our economically important marine species. Fairly extensive research is now available to evaluate the effects of chemicals on non-target freshwater and marine organisms.

Toxaphene at very high concentrations (greater than 1 ppm) is required to block the development of clam eggs, but levels of less than 0.25 ppm kill 67% of the larvae (Section 5.3.2.2.4). It is well-established that early embryogenesis is under the control of m-RNA manufactured in the ovary prior to the completion of oogenesis. These m-RNA compounds (or masked messengers, as they are called) direct the early development of the organism. Only following gastrulation are the maternal and paternal chromosomes of the new individual required to manufacture their own m-RNA. Failure of the larvae to continue development could be the result of the lack of DNA-directed RNA production and hence a loss in RNA-directed enzyme and protein synthesis. This in no way answers the question of where toxaphene is bound in the cell or what its specific activity within the cell is. However, one could suggest that the above effects might be the result of (1) an interaction with enzymes or proteins that causes a change in normal activity; (2) an interaction at the genetic level preventing genetic transcription, altering genetic transcription, or even causing a mutation; (3) an interaction of toxaphene with cellular or subcellular membranes that might mimic the above two alternatives.

Albaugh (1972) has described the symptoms of the acute toxicity response in the crayfish as hyperactivity, loss of equilibrium and coordination, paralysis, and death. Death occurred 1 to 24 hours after the symptoms were first observed. Those animals surviving at 48 hours completely recovered when transferred to clean water, even though many had displayed advanced symptoms of poisoning. These physiological responses of the organism to toxaphene seem to indicate a response that is similar to that described in higher animals (Section 6.2.2.1.1.1). These responses are thought to be of neural origin. This would seem to conflict with the information of both Metcalf (1955) and Wagner (1974); however, no specifics were given in the last two instances, only generalities without supporting experimental data. It seems cogent to surmise that toxaphene may have a neurotoxic effect in aquatic invertebrates.

5.3.2.2.1 Sublethal toxicity -- Oysters, Crassostera virginica, were exposed for four weeks to a 0.1 ppm concentration of toxaphene (Butler et al., 1962). Initially, they measured both shell movements employing a kymograph and growth of new shell in yearling oysters. It became evident to Butler et al. that measurement of shell growth was their most critical bioassay. The analysis of the activity records of the large oysters and growth of the small oysters is summarized in Figure 5.5. No growth was evident in the experimental group after the second week. The harmful effects of toxaphene were also clearly demonstrated by the 70 per cent decrease in activity of experimental oysters, as compared to controls, at the end of the fourth week of the experiment.

Sublethal concentrations were calculated in a study by Schoettger and Olive (1961) for the purpose of feeding these exposed animals to another component of the food chain. Sublethal concentrations were as follows: for Daphnia pulex, 0.03 ppm after 168 hours; for Daphnia magna, 0.03 ppm after 120 hours; and for damselfly nymphs, 0.004 ppm after 96 hours.

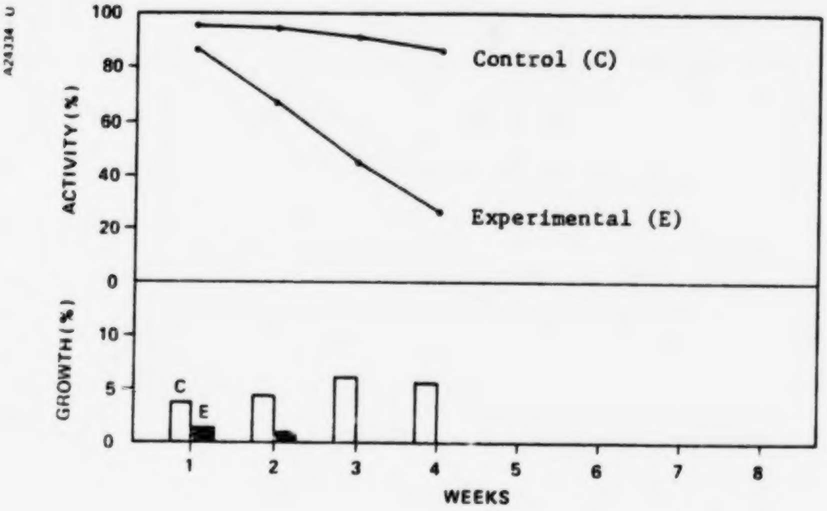


Figure 5.5. Activity of large oysters and growth of small oysters exposed to 0.1 ppm toxaphene. Source: Butler et al., 1962.

Needham (1966) determined the levels of toxaphene which numerous freshwater aquatic invertebrates would tolerate for 24 hours without any mortality. The highest concentration was 1 ppm for leeches (Hirudinea), while the least resistant organism was a Coleopteran (*Haliplus*) which would tolerate only 10 ppb. Table 5.20 gives the data for the 14 genera tested. The nine orders of invertebrates tested are listed commencing with the most tolerant order and concluding with the least tolerant: Hirudinea, Hydracarina, Gastropoda, Trichoptera, Ononata, Hemiptera, Ephemeroptera, Amphipoda, and Coleoptera.

5.3.2.2.2 *Acute toxicity* -- Khattat and Farley (1976) exposed the estuarine copepod *Acartia tonsa* Dana to toxaphene at concentrations ranging from 0.03 to 300 ppt (nanograms/liter). They calculated an LC50 of 7.20 ppt (Table 5.21).

In an extensive study by Courtenay and Roberts (1973), the toxic effects of toxaphene on *Sesarma cinereum* (drift line crab), *Penaeus duorarum* (pink shrimp), and *Rhithropanepeus harrisii* (mud crab) were examined. The 96-hour LC50 for stage I zoea in *S. cinercum* was 0.054 ppb. The LC50 increased about tenfold to 0.076 ppb for stage II zoea and to 0.74 ppb for stage III zoea. The LC50 again increased about tenfold to 6.8 ppb for stage IV zoea and to 8.4 ppb for the megalopa. Courtenay and Roberts (1973) also studied the synergistic effects of temperature, salinity, and oxygen saturation on the expression of the toxaphene toxicity. In general, higher temperatures, higher salinities, and lower oxygen saturation increased the toxicity of toxaphene to one or more of the zoeal or megalopa stages evaluated (Table 5.20).

The 96 hour LC50 for *P. durorarum* decreased from 2.2 ppb for nauplii to 1.8 ppb for protozoeae to 1.4 ppb for mysis. In this study there was a significant interaction at every stage in the temperature-toxaphene tests. A significant interaction of salinity-toxaphene was observed only in the mysis stage. The nauplius stage showed significant mortality only when toxaphene was present. Decreased oxygen levels had no effect on the toxaphene toxicity (Table 5.20).

Table 5.20. Acute toxicity of toxaphene to aquatic invertebrates

Reference	Organism	Concentration	Percent mortality	Control mortality
Khattat and Farley, 1976	Acartia tonsa Dana	300.0 ppt	77.5 (96 hr)	5
		30.0	67.5	5
		3.0	35.0	5
		0.3	27.5	5
		0.03	30.0	5
Courtenay and Roberts, 1973	Sesarma cinereum Stage I - zoeal	0.05 ppb (25 C)	0.0	0.0
		(35 C)	62.5	10.0
		(25^{o}/oo salinity)	17.5	10.0
		(34^{o}/oo salinity)	100.0	27.5
		(75^{o}/o oxygen saturation)	5.0	0.0
		(100^{o}/o oxygen saturation)	0.0	0.0
	Stage II - zoeal	0.5 ppb (25 C)	15.0	2.5
		(35 C)	70.0	22.5
		(30^{o}/oo salinity)	20.0	7.5
		(40^{o}/oo salinity)	40.0	0.0
		(75^{o}/o oxygen saturation)	87.5	20.0
		(100^{o}/oo oxygen saturation)	22.5	2.5
	Stage III - zoeal	0.5 ppb (25 C)	2.5	2.5
		(35 C)	67.8	40.0
		(30^{o}/oo salinity)	22.5	22.5
		(40^{o}/oo salinity)	45.0	32.5
		(75^{o}/o oxygen saturation)	32.5	27.5
		(100^{o}/o oxygen saturation)	10.0	22.5

Table 5.20. (continued)

Reference	Organism	Concentration	Percent mortality	Control mortality
Courtenay and Roberts, 1973	*Sesarma cinereum* Stage IV - zoeal	5.0 ppb (25 C)	27.5	35.0
		(35 C)	80.0	47.5
		(30^{o}/oo salinity)	40.0	27.5
		(45^{o}/oo salinity)	85.0	37.5
		(75^{o}/o oxygen saturation)	100.0	32.5
		(100^{o}/o oxygen saturation)	32.5	22.5
	Megalopa	5.0 ppb (25 C)	2.5	17.5
		(35 C)	10.0	17.5
		(30^{o}/oo salinity)	17.5	10.0
		(50^{o}/oo salinity)	77.5	40.0
		(75^{o}/o oxygen saturation)	5.0	7.5
		(100^{o}/o oxygen saturation)	15.0	25.0
	Penaeus duorarum nauplius	1.0 ppb (25 C)	20.0	10.0
		(35 C)	100.0	32.5
		(35^{o}/oo salinity)	17.5	0.0
		(45^{o}/oo salinity)	15.0	0.0
		(75^{o}/o oxygen saturation)	2.5	0.0
		(100^{o}/o oxygen saturation)	5.0	0.0

Table 5.20. (continued)

Reference	Organism	Concentration	Percent mortality	Control mortality
Courtenay and Roberts, 1973	*Penaeus dourarum* protozoea	1.0 ppb (25 C)	27.5	5.0
		(35°C)	97.5	37.5
		(35°/oo salinity)	22.5	22.5
		(45°/oo salinity)	40.0	25.0
		(75°/o oxygen saturation)	87.5	32.5
		(100°/o oxygen saturation)	15.0	10.0
	mysis	1.0 ppb (25 C)	30.0	15.0
		(35°C)	90.0	25.0
		(35°/oo salinity)	15.0	17.5
		(50°/oo salinity)	67.5	32.5
		(75°/o oxygen saturation)	12.5	12.5
		(100°/o oxygen saturation)	15.0	15.0
Courtenay and Roberts, 1973	*Rhithropanopeus harisii* Stage I - zoeal	10 ppb	10.0	
		20	10.0	
		30	7.0	
		40	32.0	
		50	75.0	
Naqvi and Ferguson, 1968	Six species of freshwater cyclopoids	15 ppb	0.0 } history	58.0 } little
		25	8.5 } of pesti-	56.0 } prior
		35	30.1 } cide	64.7 } pesticide
		45	28.3 } contamination	84.0 } contaminatio

Table 5.20 (continued)

Reference	Organism	Concentration	Percent mortality		Control mortality	
Naqvi and Ferguson, 1968	*Eupera singleyi*	100 ppb	0.0	history of pesticide contamination	0.0	little prior pesticide contaminatio
		200	0.0		30.0	
		300	15.0		30.0	
		400	50.0		55.0	
		500	50.0		40.0	
		600	60.0		95.0	
		700	40.0		100.0	
	Physa gyrina	350 ppb	20.0	history of pesticide contamination	80.0	little prior pesticide contaminatio
		400	60.0		75.0	
		450	35.0		100.0	
		500	55.0		100.0	
		550	100.0		100.0	
Harris and Jones, 1962	*Culicoides cariipennis* larvae	88 ppb	50.0		-	
		412	90.0		-	
Needham, 1966	Hirudinea	1 ppm	0.0		-	
	Gammarus sp.	100 ppb	0.0		9.0	
		200	61.0		9.0	
		300	79.0		9.0	
		500	100.0		9.0	
	Hydracarina	1 ppm	0.0		0.0	
	Callibaetis sp.	150 ppb	0.0		0.0	
		300	29.0		0.0	
		400	87.0		0.0	
		500	100.0		0.0	

Table 5.20 (continued)

Reference	Organism	Concentration	Percent mortality	Control mortality
Needham, 1966	*Aeschna* sp.	200 ppb	0.0	0.0
		275	16.0	0.0
		350	60.0	0.0
		450	100.0	0.0
	Lestes sp.	450 ppb	0.0	0.0
		500	19.0	0.0
		600	67.0	0.0
		850	100.0	0.0
	Notonecta sp.	275 ppb	0.0	0.0
		300	29.0	0.0
		400	61.0	0.0
		600	100.0	0.0
	Sigara sp.	50 ppb	0.0	0.0
		75	40.0	0.0
		100	75.0	0.0
		150	100.0	0.0
	Limephilus sp.	500 ppb	0.0	0.0
		550	51.0	0.0
		600	80.0	0.0
		650	100.0	0.0
	Haliplus sp.	10 ppb	0.0	0.0
		40	55.0	0.0
		50	89.0	0.0
		75	100.0	0.0

Table 5.20 (continued)

Reference	Organism	Concentration	Percent mortality	Control mortality
Needham, 1966	Hydroporus sp.	60 ppb	0.0	0.0
		100	27.0	0.0
		300	65.0	0.0
		450	100.0	0.0
	Dytiscus sp.	15 ppb	0.0	0.0
		50	24.0	0.0
		60	42.0	0.0
		75	100.0	0.0
	Gyrinus sp.	65 ppb	0.0	0.0
		100	22.0	0.0
		150	40.0	0.0
		185	100.0	0.0
	Lymnaea sp.	700	0.0	0.0

Table 5.21. LC50 values for aquatic invertebrates exposed to toxaphene

Reference	Organism	LC50
Khattat and Farley, 1976	Acartia tonsa Dana	7.2 ppt (96 hr)
Courtenay and Roberts, 1973	Sesarma cinereum	
	Stage I - zoeal	0.054 ppb (96 hr)
	Stage II - zoeal	0.760
	Stage III - zoeal	0.740
	Stage IV - zoeal	6.8
	Megalopa	8.4
	Penaeus duorarum	
	nauplius	2.2 ppb (96 hr)
	protozoea	1.8
	mysis	1.4
	Rhithropanopeus harrisii	
	Stage I - zoeal	43.75 ppb (96 hr)
	Stage II - zoeal	288-345
	Stage III - zoeal	290-430
Chaiyarach et al., 1975	Palaemonetes kadiakensis	0.091 ppm (24 hr)
		0.068 (48 hr)
		0.046 (72 hr)
		0.036 (96 hr)
	Procambarus simulans	0.45 (24 hr)
		0.29 (48 hr)
		0.24 (72 hr)
		0.21 (96 hr)
	Rangia cuneata	940 (24 hr)
		699 (48 hr)
		488 (72 hr)
		460 (96 hr)
Hooper and Grzenda, 1957	Ephemera simulans nymphs	9.5 ppm (24 hr)
	Daphnia magna	1.5
	Gammarus fasciatus	0.06
	Asellus intermedius	0.10
Naqvi and Ferguson, 1970	Palaemonetes kadiakensis	
	Bluff Lake - no history of exposure	20.9 ppb (24 hr)
	Hollandale } prior history of insecticide exposure	229.0
	Belzoni	89.0
	Sky Lake	44.0

Table 5.21 (continued)

Reference	Organism	LC50
McDonald, 1962	Gammarus lacustris lacustris	1.0 ppm (72 ± 10 min)
		0.5 (96 ± 11 min)
		0.1 (360 ± 40 min)
		0.05 (460 ± 80 min)
Albaugh, 1972	Procambarus acutus	60.7 ppb (48 hr-clean area)
		90.2 (48 hr-cottonfield area)
Ferguson et al., 1965b	Palaemonetes kadiakensis	57.5 (36 hr-clean area)
		170.0 (36 hr-cottonfield area)
Sanders and Cope, 1966	Simocephalus serrulatus	19.0 ppb (48 hr-60 F)
		10.0 (48 hr-70 F)
	Daphnia pulex	15.0 (48 hr)
Naqvi, 1973	Branchiura sowerbyi	1.0 ppm (72 hr LC100)
Sanders and Cope, 1968	Pteronarcys california naiads	18.0 ppm (24 hr)
		7.0 (48 hr)
		2.3 (96 hr)
	Pteronarcello badia naiads	9.2 ppm (24 hr)
		5.6 (48 hr)
		3.0 (96 hr)
	Claassenia sabulosa naiads	6.0 (24 hr)
		3.2 (48 hr)
		1.3 (96 hr)
Pigatti and Mello, 1961	Culex pipens fatigans larvae	14 ppm
Harris and Jones, 1962	Culicoides variipennis larvae	0.09 ppm (24 hr)
Guyer et al., 1971	Crassostera virginia	0.02 ppm (96 hr)
	Peneus aztecus	0.0027 (48 hr)
	Peneus duorarum	0.0042
	Palemonetes kadiakensis	0.006
	Palemonetes pregio	0.0052
	Palemonetes maciodactylus	0.037
	Daphnia pulex	0.015
	Daphnia serrulatus	0.01
	Gammarus fasciatus	0.022
	Gammarus lacustris	0.07

Table 5.21 (continued)

Reference	Organism	LC50
Guyer et al., 1971	*Pteronareys californía*	0.007 ppm (48 hr)
	Clossonia sabulosa	0.0032
	Pteronarcella bodia	0.0056
	Ischmura verticalis	0.086

The LC50 for stage I of R. harrisii was determined to be 43.75 ppb. Values for stages II and III ranged from 286 to 430 ppb (Table 5.21). Additionally, Courtenay and Roberts (1973) histologically evaluated S. cinereum, R. harrisii, and Callinectes sapidus (blue crab). In stage I of S. cinereum, the hepatopancreas had vacuolated granulated cells coupled with contraction of the intestine in one of the four specimens examined. In stage II, 20% of the animals exhibited no effects, 50% had the vacuolated granulated hepatopancreas cells, and 30% had necrotic spaces (undefined by the authors). Forty percent had intestinal contraction, 10% had a slight dilation, and 50% had no other apparent intestinal anomaly. In stage III all animals examined showed extensive hepatopancreas degeneration and 80% exhibited the contraction of the intestine.

Stage I C. sapidus larvae exhibited no histological changes at toxaphene concentrations of 0.0005 or 0.00001 ppb. However, 50% of the stage III larvae exposed to 0.01 ppb showed hepatopancreas changes similiar to those found in S. cinereum.

In stage I R. harrisii, one of the three specimens examined, had necrosis of the intestine and sloughing of the stomach epithelium. Stages II and IV showed damage to the nervous tissue in the form of irregular spaces between the fibers.

The authors stated that in all three species deposition of an orange-brown pigment has been found in the body spaces. The pigment deposition was especially heavy along the intestine and attempts to dissolve the pigment in water, alcohol, or xylene failed.

In a study by Chaiyarach et al. (1975), three species of aquatic invertebrates were exposed to acute levels of toxaphene. The organisms were Palaemonetes kadiakensis (grass shrimp), whose LC50 was 0.091 at 24 hours and 0.036 ppm at

96 hours; *Procambarus simulans* (crayfish), whose LC50 was 0.45 ppm at 24 hours and 0.21 ppm at 96 hours; and *Rangia cuneata* (mactrid clam), whose LC50 was 940 ppm at 24 hours and 460 ppm at 96 hours (Table 5.21). It is interesting to note that *Rangia* is the most tolerant species tested, and that it is also of commercial value as the meat is eaten by man in part of its distribution range.

In 1954 eight lakes in British Columbia were treated with "Fish Tox" - a rotenone based chemical containing 10% toxaphene (Stringer and McMynn, 1958). Two lakes each were treated with 0.01, 0.03, 0.07, and 0.10 ppm. An evaluation of the effects of toxaphene on the bottom fauna was carried out with 30 pre-poisoning and 70 post-poisoning dredgings taken from each lake. Qualitative results are summarized in Table 5.22. Shrimp (Gammarus or Hyallela) were found in six of the eight lakes prior to treatment, but were absent in subsequent sampling. Midge larvae (chironomidae) were present in all lakes initially and at one month post-poisoning. However, at nine months post-poisoning, the midge larvae were absent in the two lakes treated at the 0.10 ppm level. Aquatic earthworms (Oligochaeta) were present in all lakes, both before and after treatment. Leeches (Hirundinea), although not present in all lakes at nine months post-treatment, were present in the two 1.0 ppm lakes, indicating that these organisms were not affected by the levels of toxaphene employed in this experiment. Mayfly nymphs (Ephemeroptera) were present in six of the lakes prior to treatment. The authors stated that subsequent samples contained no nymphs except for Taylor Lake (0.01 ppm). However, their data indicated (Table 5.23) that mayfly nymphs were present in Spectacle Lake (0.07 ppm) at all three sampling periods. Dragonfly and damselfly nymphs (Odonata) were found in most lakes initially and appear to

Table 5.22. Occurrence of bottom organisms in toxaphene treated lakes before and after poisoning[a]

Organism	Lake																							
	Alleyne (0.01 ppm)			Taylor (0.01 ppm)			Gladstone (0.03 ppm)			Round (0.03 ppm)			Gallagher (0.07 ppm)			Spectacle (0.07 ppm)			Summit (0.10 ppm)			Lady King (0.10 ppm)		
	A	B	C[b]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Shrimp	0	-	-[c]	+	-	-	0	-	-	+	0	-	+	-	-	+	-	-	+	-	-	+	-	-
Midge Larvae	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	-	+	+	-
Aquatic Earthworms	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+
Leeches	-	-	-	-	-	-	+	+	-	-	0	-	-	-	-	+	-	+	+	+	+	-	-	+
Mayfly Numphs	-	-	-	+	+	-	+	-	-	+	0	-	+	-	-	+	+	+	+	-	-	-	-	-
Dragonfly and Damselfly Nymphs	+	-	-	+	+	+	+	+	+	+	0	-	+	-	-	+	+	+	+	-	-	-	-	-
Freshwater Snails	+	+	+	+	+	+	+	+	+	+	0	-	+	+	+	+	+	+	+	+	-	+	+	+

[a] Source: Modified from Stringer and McMynn, 1958.

[b] A - before treatment; B - one month after treatment; C - nine months after treatment.

[c] + indicates organism is present; - indicates organism is not present; 0 indicates that lake was not sampled.

Table 5.23. Plankton samples taken before and after toxaphene treatment[a]

Organisms	Lake																							
	Alleyne (0.01 ppm)			Taylor (0.01 ppm)			Gladstone (0.03 ppm)			Round (0.03 ppm)			Gallagher (0.07 ppm)			Spectacle (0.07 ppm)			Summit (0.10 ppm)			Lady King (0.10 ppm)		
	A	B	C[b]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Cladocera:																								
Daphnia	-	+	+[c]	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	-	-	+	+	+
Bosmina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Copepoda:																								
Cyclops	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+
Diaptomus	+	+	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	+	-	+
Nauplius larvae	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	+	+	-	-	+	+	+
Rotifera	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flagellates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diatoms	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+

[a] Source: Modified from Stringer and McMynn, 1958.

[b] A - before treatment; B - one month after treatment; C - nine months after treatment.

[c] + indicates organism is present; - indicates organism is not present.

Table 5.24. Invertebrates collected before and after treatment with 0.1 ppm toxaphene[a]

Location and date of sampling	Average number of animals per square meter						
	Diptera	Ondonata	Ephemeroptera	Oligochaeta	Mollusca	Others	Total
Horseshoe Lake							
Sept. 20 (1 day before treatment)	989.9	68.9	516.5	5.4	19.4	32.3	1678.6
Oct. 15	167.9	-	43.0	51.6	51.6	4.3	314.1
April 29	4.3	-	-	12.9	8.6	-	43.0
July 29	36.6	116.2	-	116.2	68.9	-	335.7
McCarty Lake							
Aug. 3 (1 day before treatment)	4605.3	-	12.9	-	-	4.3	464.8
Aug. 24	6.5	-	-	6.5	6.5	-	20.4
May 25	-	-	-	47.3	-	-	47.3
June 23	9856.2	-	-	-	172.2		10,028.3

[a] Source: **Modified from Hooper and Grzenda, 1957.**

be eliminated at about the 0.07 ppm level as they were absent in one lake at this level and in both 1.0 ppm lakes. Freshwater snails (Gastropoda) were taken in most dredgings and appear to be unaffected by these dosages of toxaphene.

In order to determine the effect of the various toxaphene concentrations on the planktonic groups, Stringer and McMynn (1968) qualitatively sampled the eight lakes, of which two each were poisoned with 0.01, 0.03, 0.07, and 0.10 ppm. The results are summarized in Table 5.23. Although there is some fluctuation in the planktonic groups evaluated, it appears that toxaphene had no effect at the levels employed in this study.

When Hooper and Grzenda (1957) studied two lakes in Michigan (Horseshoe and McCarty Lakes) that were treated with 0.1 ppm toxaphene, they found a large portion of the benthos was killed in a month (Table 5.24). Horseshoe Lake invertebrates seemed more affected than those from McCarty Lake. In Horseshoe Lake, the mollusks and certain oligochetes were the only groups not harmed. A few midges and mayflies survived for more than three weeks, but these species were not collected the following spring. Repopulation in Horseshoe Lake had begun by late July of the following year, with most groups of invertebrates represented. However, several aquatic diptera, e.g. Chaborus, were not present. Eleven months after treatment, McCarty Lake had a greater standing crop than existed prior to treatment. The bottom fauna was almost entirely a single species of midge (Tendipes plumasus).

In a study with the freshwater shrimp (Palaemonetes kadiakensis), Naqvi and Ferguson (1970) collected shrimp from three areas in Mississippi with a history of insecticide exposure. For comparison, they used shrimp from a wildlife refuge which had no history of agricultural pesticides. The LC50 for shrimp from the wildlife refuge was 21 ppb, and for those shrimp obtained from areas of prior pesticide exposure, the LC50 ranged from 44 to 229 ppb (Table 5.21). The Naqvi and Ferguson study (1970) and the data reported by Chaiyarach et al. (1975) agree very well on the LC50 value for P. kadiakensis (Table 5.21). Both studies collected the test animals from ditches with a history of past pesticide exposure, and the Chaiyarach study LC50 falls in the midrange of the three pre-exposed sampling areas of the Naqvi and Ferguson study, 91 and 44, 89 and 229 ppb, respectively.

McDonald (1962) studied the freshwater shrimp (Gammarus lacustris lacustris), and calculated LC50's for four concentrations of toxaphene (Table 5.21). Projection of McDonald's data out to 24 hours would give an LC50 of less than 20 ppb, which agrees with the results of Naqvi and Ferguson (1970).

Naqvi and Ferguson (1968) took six species of cyclopoid copepods, a clam (Eupera singleyi), a snail (Physa gyrina), and a tubificid worm (Tubifex tubifex) from a pesticide-contaminated drainage ditch and from a minimal pesticide-contaminated area and compared 48-hour tolerance levels to toxaphene (Table 5.20). Representative comparisons reveal that at 45 ppb the lethality to the six cyclopoids is three times greater in the non-exposed organisms than it is in the pre-exposed animals; at 700 ppb in the clam, the mortality in the pre-exposed animals is 40% as compared to 100% in the non-exposed animals; and finally at 240 ppb in the snail, the mortality in the pre-exposed animals is 35% and in the non-exposed animals is 100%.

Another incidence of acute toxicity differences due to the origin and prior exposure of the organism to pesticides is demonstrated by Albaugh (1972). This investigation involved two crayfish populations (Procambrus acutus), one population from a floodplain near cotton fields and the other in an area free from pesticides. A 1.5 increase in the LC50 was noted (Table 5.21). In comparing the LC50 of 60.7 ppb in this study for P. acutus to the LC50 of 29 ppb in Chaiyarach et al. (1975) for P. simulans, the LD50's differ by a factor of two. This could be due to the different species involved or the particular level of resistance present.

Freshwater shrimp (*Palaemontes kadiokensis*) obtained from a bayou fed by run off from cotton fields were exposed for 36 hours to determine the LC50 and were compared to shrimp from untreated areas (Ferguson et al., 1965 b). Shrimp in the bayou had an LC50 that was three times higher than in the shrimp obtained from the non-pesticide-exposed area (Table 5.21).

Sanders and Cope (1966) exposed two species of daphnids (*Simocephalus serrulatus* and *Daphnia pulex*) to toxaphene to determine the 48-hour LC50. The values for the two species are very close, varying some with temperature, but ranging from 7 to 29 ppm (Table 5.21).

Naqvi (1973) exposed the tubificid worm (*Brachiwa sowerbyi*) to 1 ppm toxaphene at three temperatures (4.4, 21, 32.2 C) and obtained 100% mortality in all groups. In the same paper it is reported that these worms were exposed to a concentration of 3.7 ppm for 9,210 mins (153 1/2 hours) and to 3.5 ppm for 7,020 mins (115 1/3 hours) with the former killing crayfish in an average of 187 mins and the latter having no effect on crayfish. It is interesting that an LC50 at 72 hours is 1 ppm, but these worms were exposed to 3.5 times this concentration of toxaphene for twice as long and lived to be fed to crayfish. Personal communication with Naqvi (1976) failed to resolve this conflict.

Sanders and Cope (1968) tested the naiads of three species of stoneflies to determine the LC50 of toxaphene at 24, 48, and 96 hours (Table 5.21). The stoneflies tested were: *Pteronarcys california*, body length of 30 to 35 mm; *Pteronarcella badia*, body length of 15 to 20 mm; and *Classenia sabulosa*, body length of 20 to 25 mm. In all of the other nine pesticides tested, the LC50 decreased with decreasing body size; however, with toxaphene the smaller naiads (*C. sabulosa*) had the intermediate LC50 (Table 5.21).

Pigatti and Mello (1961), using a technical grade of toxaphene, obtained an LC50 for mosquito larvae (*Culex pipens fatigans*) of 14 ppm (Table 5.21).

In a study by Harris and Jones (1962) with 15 insecticides, toxaphene was the least toxic chlorinated hydrocarbon to *Culicoides variipennis* larvae, having an LC50 of 0.09 ppm compared to an LC50 for heptachlor of 0.004 ppm.

The effects of toxaphene on the macroscopic bottom fauna in a Colorado lake have been investigated by Cushing and Olive (1956). The experimental lake, Reservoir No. 4, is a single basin lake with a surface area of approximately 24 ha, is 4.3 m deep, and was treated with 0.1 ppm toxaphene. The control lake, Reservoir No. 2, is a similiar lake with a depth of 2.1 m. Sampling was done every two weeks when possible. The results are summarized in Table 5.25. Application of toxaphene completely eradicated the tendipedid population. In the sample taken the day following the application, only two living organisms were found. Four days after application, large masses of dead bloodworms washed up on the shore indicating an immediate effect on the *Chironomus* larvae population. No living tendipedidae larvae were seen in the lake again until repopulation started some eight months later.

Chaoborus larvae showed no immediate effects in the experimental lake. Numbers actually increased during the winter with the larvae disappearing completely after six months. The authors speculate that the absence of an Entomostraca population, upon which the *Chaoborus* larvae are predaceous, led to the starvation of the *Chaoborus* larvae. In the control lake the population remained relatively stable during the winter and began to pupate in the spring. No specimens were found in August during the height of emergence, but were present again in September. The experimental lake failed to repopulate in September.

Table 5.25. Bottom fauna in two Colorado lakes expressed as organisms per square meter[a]

Date	Tendipedidae		Chaoborus		Palpomyia		Physa		Oligochaeta	
	exp. lake	control	exp.	control	exp.	control	exp.	control	exp.	control
Oct. 21, 1954	237	398	882	484	0	0	0	0	0	22
Oct. 26, 1954	377	1055	527	237	0	0	0	0	22	0
Poisoned Oct. 28										
Oct. 29, 1954	226 (2 alive)	-	968	-	0	-	0	-	75	-
Oct. 31, 1954	97 (dead)	-	258	-	22	-	0	-	32	-
Nov. 4, 1954	161 (dead)	-	1291	-	0	-	0	-	22	-
Nov. 11, 1954	118 (dead)	1549	1173	463	11	0	0	0	97	22
Nov. 21, 1954	32 (dead)	1291	1119	398	0	0	0	0	54	11
Dec. 22, 1954	0	-	958	-	65	-	0	-	108	-
Jan. 8, 1955	0	1463	764	495	0	0	0	0	452	11
Jan. 22, 1955	0	1517	527	409	0	0	0	0	129	11
Feb. 5, 1955	0	1302	334	377	0	0	0	0	-	43
Feb. 25, 1955	0	1775	118	355	0	0	0	0	398	22
Mar. 4, 1955	0	1711	248	721	0	0	0	0	570	32
Mar. 19, 1955	0	1582	32	592	0	0	0	0	882	11
Apr. 2, 1955	0	1291	11	323	0	0	0	0	732	0
Apr. 16, 1955	0	1517	0	581	0	0	0	0	678	0
Apr. 30, 1955	0	1270	0	312	0		0	0	1506	11
May 15, 1955	0	710	0	204	0	0	0	0	1162	11
May 29, 1955	11	409	0	280	0	0	0	0	2184	11
June 22, 1955	0	65	0	32	0	0	0	0	1367	11
July 6, 1955	151	463	0	32	0	0	0	0	1205	11
July 20, 1955	226	1377	0	11	0	0	43	0	1797	43
Aug. 3, 1955	484	861	0	0	0	0	22	11	2001	0
Aug. 24, 1955	538	118	0	0	0	0	97	0	1356	0
Sept. 14, 1955	151	129	0	151	0	0	161	0	2981	32
Sept. 30, 1955	215	420	0	194	0	22	75	0	3551	75

[a] Source: Modified from Cushing and Olive, 1956.

The oligochaeta population in the experimental lake was not adversely affected by the application of toxaphene. In fact, numbers of oligochaetae increased throughout the study, probably due to the increased organic matter that became available following the poisoning.

Needham (1966) made an extensive study on the plankton and aquatic invertebrates in four North Dakota lakes. The data from Wolf Butte Reservoir, a 9.7 ha lake with a maximum depth of 2.7 m and treated with 35 ppb, are summarized in Table 5.26. Comparison of numbers per liter was made between pre- and post-treatment collections. Rotifers (*Keratella* and *Asplanchna*) were the most numerous. *Keratella* decreased from 91 before treatment to 15 at one week, two at one month, and only one at one year post-treatment. *Asplanchna* increased from 73 to 106 at one week, but none were present at one month or at one year post-treatment. Cladocerans (*Daphnia* and *Bosmina*) were both less frequent one year after treatment. Copepoda (*Deaptomus*, *Cyclops*, and undetermined nauplii) were also less frequent one year after treatment. Total numbers and number of genera remained depressed in this group one year post-treatment.

Plant-inhabiting organisms were collected simultaneously and expressed as organisms per kilogram of vegetation (Table 5.27). *Gammarus* (an amphipoda) varied throughout the study, but remained abundant. The dominant Ephemeroptera (*Callibaetis* and *Caenis*) and Odonata (*Ischnura*) decreased at one week and one month after treatment, but were abundant at one year. The Dipteran, *Tendipes*, decreased at one week but rebounded at one month and was about at one-half its original frequency at one year. Bottom fauna was sampled at the same location as the plant-inhabiting organisms and is expressed as organisms per square meter of bottom (Table 5.27). Only two genera were abundant, *Gammarus* and *Tendipes*. *Tendipes* rebounded after a year and the *Gammarus* population seemed depressed. A comparison of these last two groups (plant-inhabiting and bottom fauna) does not reveal any shift in

Table 5.26. Number of organisms per liter in Wolfe Butte Reservoir before and after application of 35 ppm toxaphene[a]

Organism	Pretreatment	1 week posttreatment	1 month posttreatment	1 year posttreatment
Brachionus	1	4	-	3
Keratella	91	15	2	1
Lecane	-	-	-	1
Trichocerce	1	-	-	-
Chromogaster	1	2	1	-
Asplanchna	73	106	-	-
Polyarthra	7	13	1	2
Filinia	1	1	-	1
Hexarthra	3	21	3	-
Daphnia	244	18	129	28
Simocephalus	-	-	1	-
Ceriodaphnia	4	9	18	-
Bosmina	98	130	18	25
Chydorus	1	-	-	-
Diaptomus	6	-	-	2
Cyclops	46	3	11	13
Nauplii	106	26	10	73

[a] Source: Modified from Needham, 1966.

Table 5.27. Number of plant-inhabiting organisms and bottom fauna in Wolfe Butte Reservoir before and after application of 35 ppm toxaphene[a]

Organism	Pretreatment		1 week Posttreatment		1 month Posttreatment		1 year Posttreatment	
	plant[b]	bottom[c]	plant	bottom	plant	bottom	plant	bottom
Oligochaeta	-	< 11	-	< 11	-	< 11	-	32
Amphipoda:								
Gammarus	139	65	378	< 11	161	474	583	< 11
Hydracarina	11	-	< 2.2	-	4	-	51	-
Ephemeroptera:								
Callibaetis	11	11	< 2.2	-	-	-	273	< 11
Caenis	13	32	2.2	< 11	-	< 11	35	< 11
Odonata:								
Symetrum	< 2.2	-	< 2.2	< 11	-	-	7	-
Ischnura	88	54	22	11	-	43	123	< 11
Hemiptere:								
Notonecta	2.2	-	-	-	7	-	22	-
Sigara	7	-	-	-	< 2.2	-	4	-
Coleoptera:								
Haliplus	4	-	< 2.2	-	-	-	2.2	-
Copelatus	< 2.2	-	-	-	< 2.2	-	4	-
Hydroptila	-	11	-	< 11	-	-	-	-
Diptera:								
Tendipes	97	301	20	129	106	97	55	269
Probezzia	< 2.2	32	-	22	-	-	-	-
Chrysops	-	11	-	11	-	-	-	-
Gastropoda:								
Physa	271	22	715	11	343	32	1949	65
Gyraulus	1426	< 11	1720	11	2662	97	1478	11
Pelecypoda:								
Pisidium	-	32	-	11	-	86	-	43

[a] Source: Modified from Needham, 1966.
[b] Organisms per kilogram of plant.
[c] Organisms per square meter of bottom.

populations of these organisms one year after treatment, with the exception of Physa which increased more than sevenfold.

The second lake studied by Needham (1966) was Raleigh Reservoir, with a surface area of 6 ha and a maximum depth of 5.5 m, and was treated with 250 ppb followed 53 days later with 90 ppb treatment. Collections were made one day before and 11, 33, and 371 days after the first treatment; a second treatment was made 53 days after the first, and the fourth collection was 318 days after this treatment. The results of this sampling are summarized in Table 5.28. Rotifers are represented by 15 genera, but only Brachionus and Asplanchna were abundant. Brachinus decreased from 114 before to 108 at 11 days, 15 at 33 days, and to only three at 371 days. Asplanchna varied from 24 before to 194 at 11 days, 16 at 33 days, and only one at 371 days. At 371 days all rotifers were scarce, and six of the original genera were not present at all. Daphnia, Ceriodaphnia, and Bosmina were the most abundant zooplankters prior to treatment, but were almost absent one year later. The Copepods were also very depressed one year post-treatment. The author points out that the lake level fell to 1.8 m the next summer and this, coupled with the double application of toxaphene, may have contributed to the depression in zooplankters.

The plant-inhabiting and bottom fauna data are presented in Table 5.29. Several changes are notable. Reductions of Callibaetis, Caenis, Ischnura, and Tendipes may be significant; all but Caenis were abundant by 37 days post-treatment. Stringer and McMynn (1958) reported that Ephemeroptera were killed by 30 ppb. The disappearance of Sigara after treatment appears also to be related to the toxaphene level. However, the reduction of Gyraulus at 371 days after treatment may be due to the lowered water level, since Hooper and Grzenda (1957) and Stringer and McMynn (1958) found Gastropoda to be unaffected by 100 ppb toxaphene.

Table 5.28. **Numbers of organisms per liter in Raleigh Reservoir before and after treatment with toxaphene - 25 ppb initially followed by 90 ppb on day 53**[a]

Organism	Before treatment	Day 11	Day 33	Day 371
Brachionus	114	108	15	3
Keratella	13	11	9	7
Platyias	< 1	17	1	-
Lecane	< 1	2	-	-
Monostyla	1	1	-	5
Trichocerca	5	-	-	< 1
Chromogaster	1	3	-	1
Asplanchna	24	194	16	1
Polyarthra	3	15	7	1
Synchaeta	1	11	13	-
Felinia	< 1	1	-	< 1
Testudinella	1	-	< 1	-
Trochosphaera	< 1	-	1	-
Hexarthra	2	-	-	-
Conochiloides	3	11	-	-
Daphnia	65	173	57	9
Ceriodaphnia	44	156	100	1
Bosmina	314	283	50	-
Chydorus	4	17	1	-
Diaptomus	10	1	1	-
Cyclops	120	9	41	8
Nauplii	190	85	19	7

[a] Source: Modified from Needham, 1966.

Table 5.29. Numbers of plant-inhabiting organisms and bottom fauna in Raleigh Reservoir before and after treatment with toxaphene - 25 ppb initially followed by 90 ppb on day 53[a]

Organism	Before treatment		Day 11		Day 33		Day 371	
	plant[b]	bottom[c]	plant	bottom	plant	bottom	plant	bottom
Oligochaeta	-	43	-	86	-	118	-	301
Hirudinea	<2.2	-	<2.2	-	-	-	11	-
Amphipoda:								
Gammarus	68	43	689	<11	1,252	32	735	11
Hydracarina	99	-	59	-	48	-	31	-
Ephemeroptera:								
Callibaetis	145	11	20	<11	<2.2	-	132	-
Caenis	407	65	22	<11	13	-	7	-
Odonata:								
Symptrum	-	-	2.2	-	< 2.2	-	37	-
Ischnura	240	22	46	11	18	-	295	-
Hemiptera:								
Notonecta	4	-	<2.2	-	<2.2	-	11	-
Sigara	86	22	<2.2	-	-	-	-	-
Coleoptera:								
Hydroporus	4	-	15	-	13	-	18	-
Diptera:								
Tendipes	26	291	<2.2	22	2.2	65	114	764
Gastropoda:								
Physa	11	-	29	-	13	-	48	-
Gyraulus	2,559	<11	3,729	43	876	22	84	-
Pelecypoda:								
Pisidium	-	<11	-	43	-	75	-	-

[a] Source: Modified from Needham, 1966.
[b] Organisms per kilogram of plant.
[c] Organisms per square meter of bottom.

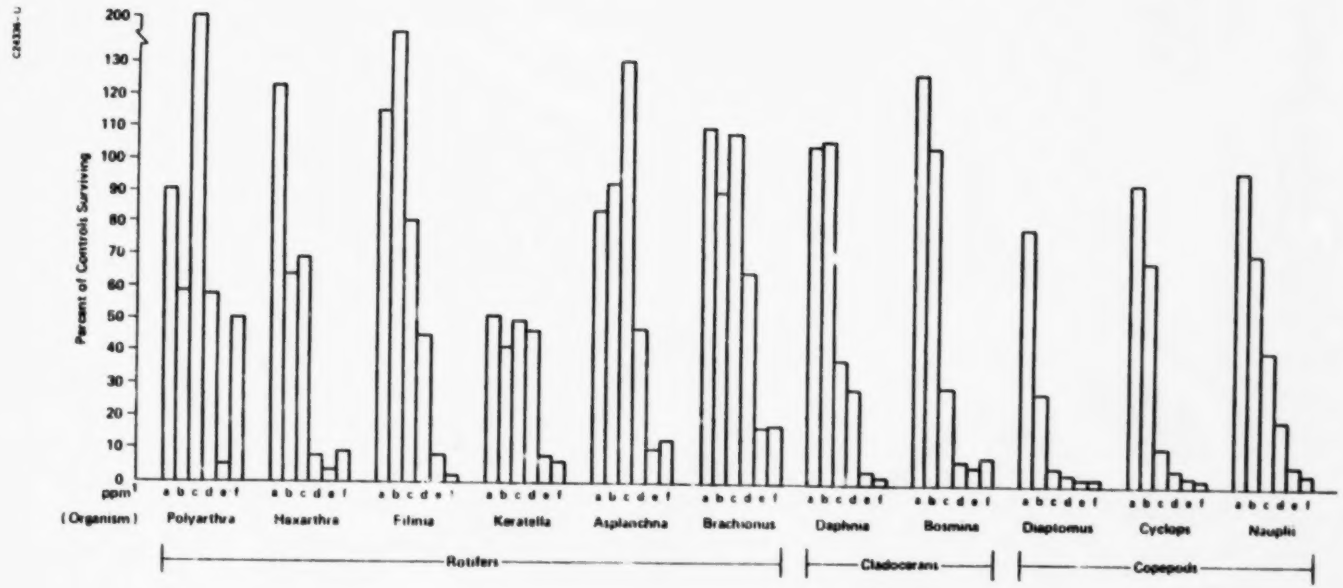
The bottom fauna was scarce in most samplings. Oligochaeta did increase, though not as dramatically as they did in the Cushing and Olive (1956) study. Likewise, the reduction in Tendipedidae may be significant as Cushing and Olive (1956) found them to be eliminated by 100 ppb.

The third lake in the study by Needham was South Lake Metigoshe, a glacial lake of 370 ha, an average depth of 2.7 m, and treated with 10 ppb followed by a 5 ppb treatment two days later. The last lake in this series was Odland Reservoir, a 40.5 ha lake with a maximum depth of 4.9 m, which was treated with 5 ppb toxaphene. The results of the last two lakes are similar. Comparisons of zooplankters before and after treatment revealed no marked changes. Likewise, comparisons of plant-inhabiting organisms and bottom fauna failed to display any numerical changes that could be attributable to the toxaphene treatment.

In a controlled laboratory experiment, Needham (1966) exposed 6 Rotifera, 2 cladocera, and 2 Copepoda to six toxaphene concentrations ranging from 50 to 1,000 ppb for 24 hours (Figure 5.6). Marked reductions in rotifers were first observed at 500 ppb, cladocerans at 250 ppb, and copepods at 100 ppb.

The large invertebrates were exposed to concentrations at which 100 percent survived for 24 hours and 100 percent died within 24 hours (Table 5.20). The Coleopteran Haliplus was the most sensitive of the species tested and the Hirudinean was the most resistant.

In an attempt to isolate the toxic components of toxaphene, Nelson and Matsumura (1975 a) have chlorinated exo-2,10-dichlorobornane to form what they term a simplified toxaphene. They concluded, in their bioassay system which included LC50 determinations in both brine shirmp and mosquito larvae, that the degree of chlorination which was optimum for toxicity was approximated by their chlorination product 3 (Table 5.30). Chlorination product 3 has approximately



[1] ppm - a = 50, b = 100, c = 250, d = 500, e = 750, f = 1000.

Figure 5.6. Effects of six levels of toxaphene on survival in selected aquatic invertebrates. Source: Needham, 1966.

Table 5.30. Toxicities to brine shrimp and mosquito larvae of chlorination products of exo-2,10-dichlorobornane[a]

Chemical	Brine shrimp Artemia salina LC50 (ppm)	Mosquito larvae Aedes aegypti LC50 (ppm)
exo-2,10-Dichlorobornane	> 4.00	> 10.00
Chlorination product 1	0.90	3.50
Chlorination product 2	0.34	0.65
Chlorination product 3	0.27	0.32
Chlorination product 4	0.26	0.63
Toxaphene	0.24	0.11

[a] Source: Modified from Nelson and Matsumura, 1975 a.

the same GC pattern as toxaphene. In an effort to clean up chlorination product 3, a TLC preparation followed by eluting with hexane on a Florisil column was done. Sixty-five fractions were then collected from the Florisil column. Florisil fractions 13, 14, and 15 gave a major peak with a retention time that corresponds to a toxic compound isolated from toxaphene earlier by Nelson. A preparative GC of the fractions isolated a peak which was checked for purity in their bioassay system. The LC50 of this preparation was 0.057 ppm for the mosquito larvae and 0.32 ppm for the brine shrimp. These values compare favorably to toxicity values for the toxic component, isolated from toxaphene, which were 0.056 ppm for the mosquito larve and 0.32 ppm for brine shrimp.

Using a detailed combination GC, TLC, and reverse phase thin-layer chromatography (RPTLC), Nelson and Matsumura (1975 b) isolated a toxic fraction from toxaphene which is 1.87 times more toxic to mosquito larvae and 1.75 times more toxic to brine shrimp (Figures 5.7 and 5.8). This fraction behaves as a single component in various chromatographic systems, but was found to consist of two components on the basis of NMR. These components have been characterized as octochlorobornanes.

Finally, in a status report to the Environmental Protection Agency, Guyer et al. (1971) listed LC50's for a variety of aquatic invertebrates. These values are presented in Table 5.21. The lowest LC50 reported was for the shrimp (Peneus aztecus) at 2.7 ppb, whereas the highest LC50 reported was 0.086 ppm for the damselfly (Ischmura verticalis).

In summary, acute toxicities vary greatly among aquatic organisms. Acartia tonsa (Khattat and Farley, 1976) has an LC50 of 7.2 ppt, whereas Tubifex tubifex (Naqvi and Ferguson, 1968) has an LC50 of greater than 6 ppm. The LC50 varies with stages of development (Section 5.3.2.2.4), salinity, pH, and - perhaps most importantly - with prior exposure to toxaphene and/or other pesticides (Section 5.3.2.3).

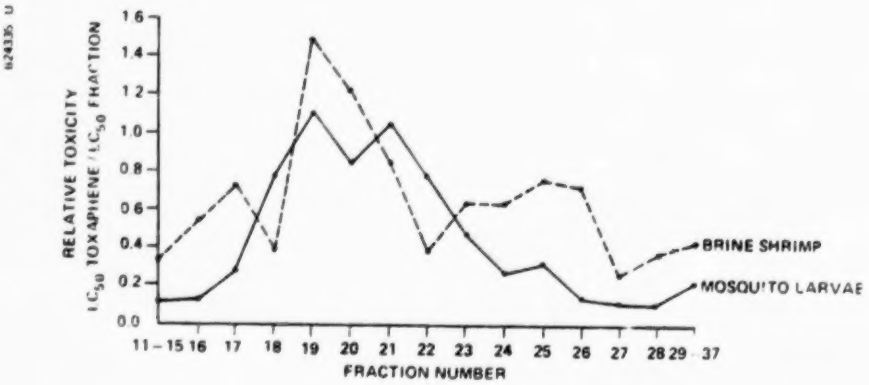


Figure 5.7. Graph of the relative toxicities of Sephadex LH-20-methanol column fractions to mosquito larvae and brine shrimp. Source: Nelson and Matsumura, 1975 b.

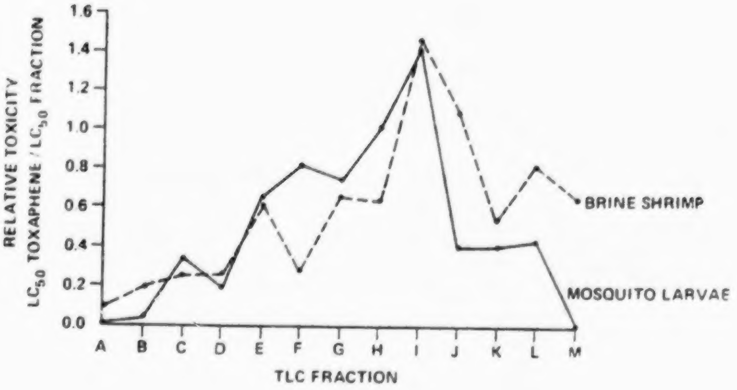


Figure 5.8. Graph of the relative toxicities of TLC separated fractions to mosquito larvae and brine shrimp. Source: Nelson and Matsumura, 1975 b.

5.3.2.2.3 Chronic toxicity -- Oysters (Crassostera virginica) grown in a mixture of 1.0 ppb each of DDT, toxaphene, and parathion were studied by Lowe et al. (1971). A significant depression in weight following the nine-month exposure period was recorded. Weights of oysters exposed to only one of these chemicals at 1.0 ppb for nine months were not significantly different from the controls. Bioaccumulation of toxaphene was noted in this study (Section 5.3.1). During the three-month depuration period which followed, the oysters were able to eliminate the pesticides to a non-detectable level (<0.010 ppm). Histologically, oysters raised in the pesticide mixture had tissue changes associated with the kidney, visceral ganglion, gills, digestive tubules, and tissues beneath the gut. A mycelial fungus invaded and caused lysis of the mantle, gut, gonads, gills, visceral ganglion, and kidney tubules. Some of the tissues showed an intense inflammatory reaction with leucocytic infiltration. Oysters observed after the recovery period showed very little pathology. Oysters exposed to toxaphene alone showed lysed Leydig cells, increased incidence of pigmented cells, and pathology of the viseral ganglion and adductor muscle.

A study on the bottom fauna and zooplankton in a drainage basin in northern Alabama, contaminated with toxaphene and benzene hexachloride, was conducted by Grzenda et al. (1964). The amount of toxaphene present in the water was measured by a carbon absorption method which was about 45% efficient for its recovery of the compound. The mean seasonal recoveries for toxaphene from the Summer of 1959 through the Fall of 1960 ranged from 29 to 140 ppt. These authors concluded that there was no convincing evidence that continuous toxaphene contamination at these levels influenced the zooplankton or bottom fauna populations. In their study they evaluated eight sampling sites at different sampling times. At only one station did the variety of organism change during the course of the year, and this was at a time following the application of insecticide in the area. This site was 2 to 6 km downstream from an area that was poisoned by fish poachers. A drop in the number of genera present from 38 to 24 followed. The chronic presence of toxaphene in the Flint Creek drainage basin in northern Alabama appears to have little effect on the number of organisms present at levels of approximately 70 to 300 ppt.

5.3.2.2.4. Embryotoxic effects -- In a study by Davis (1961), in which he exposed clam eggs and larvae (*Venus mercenaria*) to toxaphene concentrations of 0.25, 0.50, 1.0, 2.5, 5.0, and 10.0 ppm, the larvae were more susceptible to the toxic effects than the eggs. Some eggs developed normally in concentrations up to 5.0 ppm. Since the solubility of toxaphene is presumably 1.5 ppm, the increasing toxicity of clam eggs at 2.5, 5.0, and 10.0 ppm possibly indicates that some of the other components of this mixture may reach toxic levels above the 1.5 ppm range. However, clam larvae exposed to toxaphene at levels of 0.25 ppm had their growth reduced drastically and caused 50% mortality. Some larvae survived for 12 days in the 0.50 ppm concentration, but growth of these larvae was negligible (Figure 5.9). Davis and Hidu (1969), in what appears to be a repeat of the Davis (1961) work, obtained results that are essentially identical in the percentage of eggs developing through normal embryonic stages into straight-hinge larvae. The larvacidal effect of toxaphene in the second study was greater, as seen by 0.25 ppm toxaphene causing 50% mortality in the first study and 67% mortality in the second. At 0.50 ppm, mortality values of 80% and 89%, respectively, were obtained. Concentrations of 1.00 ppm and greater prevented development in both studies. Davis and Hidu (1969) took their data and calculated a 48-hour TLm (the concentration in ppm that would cause an approximate 50% reduction in the number of eggs developing into normal, straight-hinge larvae). They calculated this to be 1.2 ppm. A similar calculation for the earlier report gives the same TLm. They also listed a 12-day TLm for the larvae to be < 0.25 ppm. The first report would give a 12-day TLm of 0.25 ppm. From these data it is apparent that toxaphene permitted development of clam eggs at concentrations considerably higher than those at which the larvae could survive and grow. The possible biochemical significance of this is discussed in Section 5.3.2.1.

5.3.2.3 Resistance/Tolerance -- The mechanism of tolerance to insecticides may take one or more forms. Rapid epoxidation of organochlorine compounds and the presence and action of dehydrochlorinases are possible mechanisms, as well as mechanical exclusion. Naqvi and Ferguson (1970), using the freshwater shrimp, demonstrated an acquired tolerance when determining the LC50 (Section 5.3.2.2). Shrimp collected from the Noxubee Refuge in Mississippi had a tolerance to toxaphene which was more than 10 times less than shrimp obtained from a drainage ditch bisecting an extensive area of cotton fields; approximately 3.5 times less than shrimp obtained from a 2024 sq. m pond receiving drainage from several cotton fields; and about 1.8 times less than shrimp obtained from a 2.6 sq. km. lake also receiving runoff from numerous cotton fields.

Naqvi and Ferguson (1968) demonstrated that freshwater cyclopoids, clams, and snails that have been exposed to pesticides previously are more tolerant than are the same species without prior exposure. Albaugh (1972) reports the same increase in tolerance in crayfish (Procambarus acutus) collected from a floodplain in Texas carrying a heavy pesticide load.

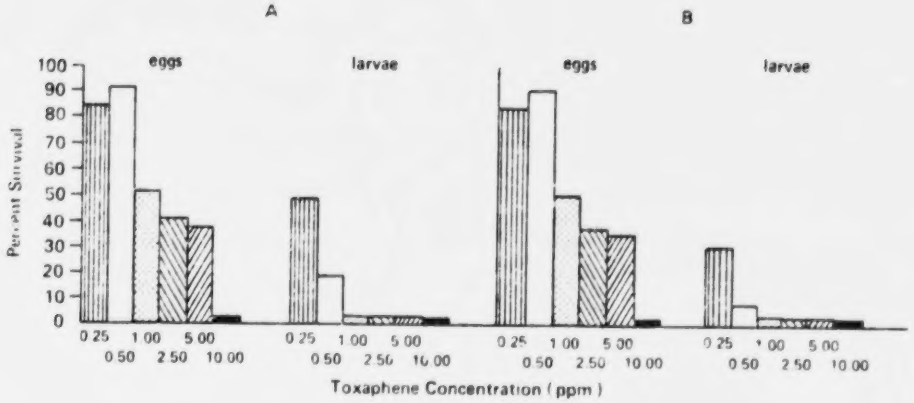


Figure 5. 9. The effects of several concentrations of toxaphene on clam eggs and larvae. Source: A. Modified from Davis, 1961. B. Modified from Davis and Hidu, 1969.

The ability of these animals to tolerate and store larger body burdens is of ecological significance. Many of these organisms tested contribute significantly to the biomass of the trophic levels upon which vertebrates are dependent. The ability of these lower invertebrates to develop resistance/tolerance, carry heavier body burdens, and survive in heavily pesticide-treated areas is a situation to be further evaluated.

Naqvi and Ferguson (1968, 1970) suggest a genetic mechanism is involved, and that this tolerance is indeed resistance. These assumptions are based in part on other work of Ferguson (Boyd and Ferguson, 1964; Burke and Ferguson, 1969; Ferguson et al., 1964, 1965 a) on mosquito fish (Section 5.2.2.2.4). Although this possibility has not been experimentally tested, the suggestion that invertebrates can develop resistance to toxaphene is strong. The possibility of biomagnification at higher trophic levels and the subsequent physiological and biochemical alterations that could occur are areas to be closely monitored.

5.4 BIRDS AND TERRESTRIAL WILDLIFE

5.4.1 Metabolism

5.4.1.1 Absorption -- No data are available.

5.4.1.2 Transport and Distribution -- No data are available.

5.4.1.3 Biotransformation -- No data are available.

5.4.1.4 Elimination -- No data are available.

5.4.1.5 Residues -- The detection of chlorinated hydrocarbon insecticide residues in tissues of numerous wildlife species has been a well-documented observation for many years. In particular, studies to determine the extent of toxaphene residue accumulation in wildlife have been undertaken by several investigators. Causey and coworkers (1972) recently collected and analyzed samples of edible meat from bobwhite quail (*Colinus virginianus*), swamp and cottontail rabbits (*Sylvilagus aquaticus* and *S. floridanus*), and white-tailed deer (*Odocoileus virginianus*). Animals were obtained either in, or adjacent to, insecticide-treated soybean fields in Alabama, and also from areas with little or no history of organochlorine insecticide treatment. Results of tissue analyses in animals collected from treated soybean fields are presented in Table 5.31. These results indicate that toxaphene was detectable in 5 of 20 quail in amounts ranging from 10.30 to 88.90 ppm (average residue level = 32.24 ppm). Two of 31 test rabbits and 3 of 22 test deer also contained toxaphene residues. Among 21 deer, 17 rabbits, and 17 quail collected from untreated areas, toxaphene residues were detected (1.26 ppm) only in a composite sample from three deer.

Chemical analyses for toxaphene in selected tissues of ring-necked pheasants were conducted by Genelly and Rudd (1956 a). Groups of six-month

Table 5.31. Toxaphene residues in bobwhite quail, rabbits, and white-tailed deer collected from treated soybean fields in Alabama during 1968 - 1969[a]

	Residue (ppm lipid basis)[b]		
Animal No.	Quail	Rabbit	Deer
1	T	* (composite of three)	*
2	10.30	* (composite of four)	*
3	88.90	*	*
4	*	* (composite of six)	*
5	26.50	*	*
6	*	*	*
7	17.40	12.35	*
8	18.75	*	*
9	*	*	*
10	*	*	*
11	*	*	*
12	*	*	*
13	*	*	*
14	*	*	*
15	*	*	1.70
16	*	1.20	*
17	*	*	*
18	T	*	*
19	T	*	6.04
20	*	*	8.70
21		*	*
22		*	*
23		*	

[a] Source: Causey et al., 1972.

[b] T = < 0.01 ppm; * = undetected at approximately 0.001 ppm level of sensitivity.

old pheasants were exposed to various levels of toxaphene in the diet and sacrificed after 74 days. Tissue samples were taken, extracted with benzene, analyzed for organic chloride content, and calculations for toxaphene concentrations were made by comparison with normal organic chloride content in control birds. Their results are summarized in Table 5.32.

During the years from 1960 to 1962 an unusually high mortality among fish-eating birds was noted in the Tule Lake Basin of California and Oregon (Keith, 1965). It was suggested that runoff from surrounding agricultural areas containing organochlorine insecticides, including toxaphene, may have been responsible for the high incidence of death. Toxaphene was used in the region between 1959 and 1960 at application rates of two pounds per acre. Analyses of dead birds were made for tissue residues of toxaphene and these results are summarized in Table 5.33. Although he pointed out that interpretation of the above results is difficult, the author suggested that ingestion by birds of 1 to 5 ppm organochlorines in the diet may result in tissue accumulation of 20 to 50 ppm after several months.

Keith (1965) extended his study of pesticide effects on fish-eating birds to attempt a correlation between mortality and tissue residues of toxaphene. Data were obtained on toxaphene residues in dead birds where death might be attributed to the pesticide exposure due to the close association in space and time between toxaphene applications and bird mortality. Toxaphene residue levels found in dead birds are presented in Table 5.34.

Additional data on toxaphene residues in birds have been summarized by Markley (1974). In his report, a study by Finley (1960) was cited involving the application of toxaphene at 1.7 kg/ha for grasshopper control. Birds

Table 5.32. Toxaphene residues in ring-necked pheasant tissues[a]

Concentration in total diet (ppm)	Sex	Experimental period (days)	Tissue	Concentration of chemical in tissue (ppm)
89	Female	74	Liver	0.0
			Fat	292.0
89	Male	74	Liver	0.0
			Testis	1,510.0
243	Female	74	Liver	6.1
243	Male	74	Liver	0.0
			Testis	0.0
243	Female	74	Liver	4.6
			Fat	752.0
Control	Female	74	Liver	0.0
			Fat	0.0

[a] Source: Modified from Genelly and Rudd, 1956 a.

Table 5.33. Toxaphene residues in fish-eating birds found dead at Tule Lake and Lower Klamath Refuges between 1960 and 1962[a]

Species	Year collected	No. of samples	Tissue sampled[b]	Average residues (ppm) toxaphene
White Pelican	1960	1	Carcass	4.0
		3	Liver	8.0
		3	Kidney	10.3
	1961	12	HLKM	7.6
	1962	2	HLKM	N.D.[c]
		1	Brain	N.D.
		5	HLKMB	N.D.
Western Grebe	1960	6	Carcass	0.3
		2	Adipose	31.5
	1962	2	HLKM	N.D.
		1	Brain	N.D.
American Egret	1960	3	Carcass	9.2
	1962	1	HLKMB	N.D.
Great Blue Heron	1960	1	Carcass	10.0
Black-crowned Night Heron	1960	1	Carcass	N.D.
	1961	1	Carcass	15.0
	1962	1	HLKMB	N.D.
Double-crested Cormorant	1961	1	Carcass	9.5
Californian Gull	1961	3	HLKM	N.D.
Ring-billed Gull	1961	1	HLKMB	N.D.
	1962	1	HLKMB	N.D.

[a] Source: Keith, 1965.

[b] Carcass samples were aliquots from whole, skinned birds passed through a meat grinder. HLKMB indicates samples consisted of a composite of 5 g each of heart, liver, kidney, breast muscle and brain, respectively.

[c] N.D. indicates no residues detected.

Table 5.34. Toxaphene residues in dead birds found in treated habitats[a]

Habitat	Level of exposure	Species[b]	Residues (ppm)
Nebraska Lake	0.05 ppm	Blue-winged Teal (3)	7
		Shoveller (1)	12
		Sandpiper (1)	10
Nebraska Lake	0.40 ppm	Black-crowned Night Heron (1)	64
		Coot (1)	17
		Mallard (1)	10
Montana Upland	1.6 - 2.2 kg/ha	Meadowlark (2)	13
		Meadowlark (3 young)	3
		Wilson's Phalarope (4)	41
		Western Kingbird (1 young)	4
		Killdeer (2)	6
		House Wren (2)	41
		Brewer's Blackbird (1)	5

[a] Source: Keith, 1965.

[b] Analyses were of aliquots of whole, skinned birds; numbers of individuals are shown in parentheses.

were analyzed for toxaphene residues in their tissues with the following results: meadowlark, 12 to 67 ppm; meadowlark nestling, 8 ppm; Wilson's phalarope, 70 to 265 ppm; killdeer, 6.44 ppm; adult house wrens, 165 ppm; and one Brewer's blackbird, 19 ppm. Table 5.35 presents further data from Markley (1974) on toxaphene residues in birds.

The passage of toxaphene into the eggs of wild birds has been documented by Keith and Hunt (1966). The results of egg sample analyses for toxaphene residues as summarized by Markley (1974) are presented in Table 5.36.

5.4.2 Effects

5.4.2.1 Physiological or Biochemical -- A recent report by Hurst et al. (1974) has presented the first data on the effects of toxaphene on thyroid physiology. These investigators exposed bobwhite quail to toxaphene at 5, 50, or 500 ppm in their feed for four months. At monthly intervals, treated birds were selected (male and female) and administered a tracer dose of I-131 by intraperitoneal injection. The birds were sacrificed after two hours and measurements were made of thyroid, adrenal, and liver weight, and thyroid radioactivity. Results are presented in Tables 5.37 and 5.38. Data from toxaphene-treated birds were compared with results obtained using untreated controls by the students' "T" test.

The data presented above suggest that thyroid weight expressed as percent of body weight (mg percent, Table 5.37) is elevated by toxaphene treatment after the fourth month. However, significant increases in I-131 uptake into the thyroid were noted only in the group treated at the highest dosage. The authors postulated that toxaphene may increase the release of thyroid stimulating hormone, which would subsequently enhance the growth of the thyroid

Table 5.35. Toxaphene residues in bird tissues[a]

Species	Tissues analyzed[b]	No. of specimens	Range or avg. of residues (ppm)	Literature citation
Cowbird, Brown-Headed Molothrus ater	WB-found dead	1/1	0.98	Hillen, 1967
Dove, Mourning Zenaidura macroura	WB-found dead	1/1	Trace	Hillen, 1967
Egret, Common Casmerodius albus	WB	1/1	17.0	DeWitt et al., 1962
Grebe, Western	Fat	5 samples	0.0 - 39.0 Avg. = 12.66	Keith and Hunt, 1966
Gull, Ring-Billed Larus delawarensis	Fat	1 sample	4.8	Keith and Hunt, 1966
Heron, Black-Crowned Night Nycticorax nicticorax	WB	3 samples	0.0 - 15.0 Avg. = 5.0	Keith and Hunt, 1966
Heron, Great Blue Ardea herodias	WB	1/1	10.0	DeWitt et al., 1962
Killdeer Charadrius vociferus	WB-found dead	1/1	9.6	Hillen, 1967
Lark, Horned Eremophilia alpestris	WB-sacrificed	4/4	0.41 - 0.96 Avg. = 0.7	Hillen, 1967
	WB-found dead	3/3	Trace, 2.5, 3.3	Hillen, 1967
Meadowlark, Western	WB-found dead	3/3	Trace, Trace, 0.6	Hillen, 1967
Pelican, White Pelecanus erythrorhyncos	Liver; Kidney	1/1	8.0, 13.0	DeWitt et al., 1962
	Liver; Kidney	1/1	9.0; 14.0	DeWitt et al., 1962
	Heart; Liver; Kidney; Breast	49 samples	0.0 - 82.0 Avg. = 3.6	Keith and Hunt, 1966
Shrike, Loggerhead Lanius ludovicianus	WB-sacrificed	1/1	Trace	Hillen, 1967

[a] Source: Markley, 1974.
[b] WB = whole body.

Table 5.36. Toxaphene residues in bird eggs[a]

Species	Tissues analyzed	No. of analyses	Range or avg. of residues (ppm)	Literature citation
Cormorant, Double-Crested Phalacrocorax auritus	Yolk	2	10.0	Keith and Hunt, 1966
Duck, Gadwall Anas strepera	Yolk	5	Avg. 0.04	Keith and Hunt, 1966
Gull, Ring-Billed Larus delawarensis	Yolk	1	0.2	Keith and Hunt, 1966
Pelican, White Pelecanus erythrorhynchos	Egg	22	0.0 - 6.7 Avg. 0.39	Keith and Hunt, 1966
Tern, Forster's Sterna forsteri	Yolk	1	15.5	Keith and Hunt, 1966

[a] Source: Markley, 1974.

Table 5.37. The effects of toxaphene on thyroid size and 2-hour uptake of I^{131} by the thyroid of Bobwhite quail[a]

Toxaphene treatment (ppm)	Time of treatment (months)	No. of animals	Mean thyroid wt. (mg)	Mean thyroid wt. (mg%)	Mean 2-hr. uptake of I^{131}, percent of injected dose	
					by total thyroid	per mg of thyroid
Control	1	6	10.1 (1.1)[b]	4.6 (0.6)	7.24 (0.72)	0.75 (0.09)
	2	9	10.6 (1.8)	5.7 (0.7)	8.60 (1.2)	0.90 (0.15)
	3	10	9.4 (1.9)	4.6 (1.0)	6.00 (0.8)	0.81 (0.10)
	4	10	6.7 (0.5)	3.6 (0.2)	7.40 (0.7)	1.18 (0.17)
5	1	6	12.6 (1.4)	6.2 (0.9)	7.18 (0.96)	0.60 (0.10)
	2	10	8.7 (0.9)	4.4 (0.5)	7.40 (0.8)	0.91 (0.09)
	3	10	9.4 (0.5)	5.0 (0.3)	5.70 (0.8)	0.62 (0.09)
	4	10	9.1 (1.1)	5.9 (0.7)**	6.90 (0.7)	0.83 (0.10)
50	1	6	14.0 (2.4)	6.7 (1.0)	8.59 (1.18)	0.66 (0.10)
	2	8	10.4 (1.1)	5.5 (0.5)	7.80 (1.0)	0.75 (0.09)
	3	10	10.2 (1.6)	5.8 (0.7)	6.80 (0.9)	0.73 (0.11)
	4	10	8.6 (0.5)	5.2 (0.5)*	8.40 (1.4)	1.02 (0.20)
500	1	6	7.1 (1.2)	5.4 (0.7)	6.47 (1.35)	0.97 (0.23)
	2	9	7.2 (0.4)	5.2 (0.3)	10.80 (0.9)	1.51 (0.12)**
	3	9	8.5 (1.3)	5.8 (0.6)	9.70 (0.8)**	1.29 (0.18)*
	4	4	9.3 (1.1)	6.2 (0.3)***	9.80 (0.6)*	1.10 (0.13)

[a] Source: Hurst et al., 1974.
[b] Numbers in ( ) = Standard error of the mean.
*Significantly different from the controls at the 0.05 level.
**Significantly different from the controls at the 0.01 level.
***Significantly different from the controls at the 0.001 level.

Table 5.38. The effects of toxaphene on body weight, adrenal weight, and liver weight of Bobwhite quail[a]

Toxaphene treatment (ppm)	Time of treatment (months)	No. of animals	Mean body wt. (gm)	Mean adrenal wt. (mg%)	Mean liver weight	
					gm	gm%
Control	1	6	226 (16)[b]	9.2 (1.6)	3.82 (0.60)	1.75 (0.32)
	2	10	175 (12)	9.8 (0.7)	2.67 (0.29)	1.53 (0.11)
	3	10	208 (5)	8.5 (0.8)	3.60 (0.30)	1.72 (0.13)
	4	10	190 (6)	8.2 (0.9)	3.28 (0.32)	1.73 (0.16)
5	1	6	210 (11)	7.7 (0.6)	3.04 (0.43)	1.43 (0.15)
	2	10	199 (7)	8.4 (0.9)	3.50 (0.28)	1.75 (0.12)
	3	10	187 (3)**	10.1 (0.6)	3.60 (0.35)	1.92 (0.17)
	4	10	157 (9)*	11.9 (0.7)**	2.57 (0.18)	1.64 (0.09)
50	1	6	211 (11)	8.6 (0.6)	3.60 (0.53)	1.67 (0.17)
	2	10	190 (4)	10.7 (1.4)	3.46 (0.19)*	1.83 (0.09)*
	3	10	175 (12)*	11.2 (2.3)	3.26 (0.35)	1.89 (0.19)
	4	10	171 (9)	10.7 (1.4)	3.35 (0.28)	1.96 (0.14)
500	1	6	130 (9)***	9.4 (1.3)	3.47 (0.37)	2.81 (0.47)
	2	10	138 (4)**	11.2 (0.5)	2.26 (0.09)	1.64 (0.07)
	3	9	147 (8)***	12.2 (1.6)	2.72 (0.25)*	1.84 (0.09)
	4	4	149 (11)*	9.9 (1.2)	2.89 (0.57)	1.90 (0.23)

[a] Source: Hurst et al., 1974.
[b] Numbers in ( ) = Standard error of the mean.
*Significantly different from the controls at the 0.05 level.
**Significantly different from the controls at the 0.01 level.
***Significantly different from the controls at the 0.001 level.

and uptake of iodide. Moreover, as the thyroid enlarged and increased its production of thyroid hormone, thyroid stimulating hormone secretion would decline accompanied by a reduction in iodide uptake. As Table 5.37 points out, each mg of thyroid tissue after four months of treatment is taking up only as much iodide as the controls. Alternatively, it was suggested that toxaphene may decrease the circulating half-life of thyroid hormone and thereby cause a compensatory hypertrophy of the thyroid.

Toxaphene treatment at the highest dosage level significantly depressed the body weight gain of birds at all time intervals. A similar, but not statistically significant, trend was noted at the other two dosages. Differences in adrenal and liver weights among treated and control birds were not generally significant. It appeared, however, that some adrenal hypertrophy may have resulted from the toxaphene treatment after three months.

The authors did not attribute specific deleterious effects to altered thyroid function in bobwhite quail. It was noted, however, that aberrant thyroid function may be associated with increased reproductive failure and abnormalities. Substantial scientific evidence exists to indicate that chlorinated hydrocarbons in general can disrupt reproductive function in many wildlife species.

An unusual report concerning the pharmacologic actions of toxaphene on a Bengal tiger has recently been published (Peavy, 1975). In this case, a female Bengal tiger, age 12 years, became poisoned after ingesting portions of a llama calf that had died after being dipped in a toxaphene solution two days prior to its death and subsequent feeding to the tiger. The tiger presented clinical symptoms identical to those produced by acute chlorinated hydrocarbon poisoning. These included periodic convulsions and hyperreflexia to sudden auditory and visual, but not tactile, stimuli. Effective control of the symptoms was obtained by administration of diazepam and methocarbamol.

5.4.2.2 Toxicity

5.4.2.2.1 Acute -- The effects of acute exposure to toxaphene have been determined in a variety of wildlife species. These investigations have generally taken the form of follow-up studies to assess the impact on wildlife from insecticide application programs. Summarizations of acute toxicity data from studies conducted under controlled experimental conditions have been prepared by Pimentel (1971) and Markley (1974). These results are presented in Table 5.39. Most acute toxicity studies with wildlife are difficult to interpret due to inadequate presentation of data and/or deficiencies in experimental design (e.g., number of animals, age, sex, statistical analysis). A careful examination of the experimental results as reported, however, should allow for a relatively accurate assessment of potential hazards to wildlife which occur by accidental exposure to toxaphene.

Dahlen and Haugen (1954) exposed bobwhite quail (8 weeks old) and mourning doves to toxaphene by oral administration in gelatin capsules. Where death occurred from the treatment, it was usually preceded by a lack of motor coordination and depression. Table 5.40 presents a summary of their experimental data, including an approximate LD50 for each species. The method of data tabulation used in this study is of questionable reliability and the authors did not provide statistical limits of significance for the calculations presented.

It was shown in a study conducted by Hudson and coworkers (1972) that the susceptibility of mallard ducks to toxaphene poisoning is an age-dependent

Table 5.39. Acute toxicity of toxaphene to birds and terrestrial wildlife

Species	Toxaphene[a] exposure	Route of administration	Effects	Reference
Mallard duck (3-5 mo., female)	70.7	oral (capsule)	LD_{50}, no other symptoms reported	Tucker and Crabtree, 1970
Pheasant (3-mo., female)	40.0	oral (capsule)	LD_{50}, no other symptoms reported	" "
Bobwhite quail (3-mo., male)	85.4	oral (capsule)	LD_{50}, no other symptoms reported	" "
Sharp-tailed grouse (1-4 yr., male)	10-20	oral (capsule)	LD_{50}, no other symptoms reported	" "
Fulvous tree duck (3-6 mo., male)	99.0	oral (capsule)	LD_{50}, no other symptoms reported	" "
Lesser sandhill crane (female)	100-316	oral (capsule)	LD_{50}, no other symptoms reported	" "
Pheasant (2-wk. old)	500-550 ppm	diet - 5 days	LC_{50} (5 day treatment followed by clean food for 3 days)	Heath et al., 1970
Coturnix (2-wk. old)	600-650 ppm	diet - 5 days	LC_{50} (5 day treatment followed by clean food for 3 days)	" "
Bobwhite quail (chick)	834 ppm	diet - 5 days	LC_{50} (5 day treatment followed by clean food for 3 days)	Heath and Stickel, 1965
Mallard duck (duckling)	563 ppm	diet - 5 days	LC_{50} (5 day treatment followed by clean food for 3 days)	" "
Mule deer (16-17 mo., male)	139-240	oral (capsule)	LD_{50}, no other symptoms reported	Tucker and Crabtree, 1970
White pelican (young)	100-400	oral	one bird died at 100 mg/kg, another was unaffected at 200 mg/kg, and a third was intoxicated at 400 mg/kg but survived	Keith, 1964
Chuckar partridge	50	?	LD_{50}, no other symptoms reported	Post, 1949
Pheasant	200	?	LD_{50}, no other symptoms reported	" "
Sage grouse	90	?	LD_{50}, no other symptoms reported	" "
White pelican (young)	100	oral	two of three birds died; survivor recovered after 48 hours	Keith, 1965
White pelican	50	oral	two of three birds died; survivor recovered after 48 hours	Keith, 1965

[a] Units of exposure are mg/kg body weight except where indicated as ppm in the diet.

Table 5.40. Toxicity data for birds exposed to toxaphene[a]

Species	No. birds	No. birds dying	LD_{50} (mg/kg)	Avg. wt. loss (%)	Avg. no. of days lived
Bobwhite Quail	60	22	80 - 100	25	3
Mourning Dove	8	5	200 - 250	22	3

[a] Source: Modified from Dahlen and Haugen, 1954.

phenomenon. Birds of both sexes were treated orally with a single dose of toxaphene and resulting LD50's were calculated (Table 5.41). Previous tests conducted by the authors had demonstrated that nonbreeding birds do not display significant sex-dependent differences in sensitivity to pesticide effects. The investigators obtained a biphasic age-sensitivity curve to toxaphene, as well as to three other central nervous system stimulants. It was suggested that the relative insensitivity of the youngest birds was due to the functional immaturity of the CNS; young animals were either unable to undergo full clonic convulsions or possibly they could tolerate higher brain concentrations of the chemical. Increasing sensitivity as the CNS matures is evident by the decreased LD50 in birds at age 7 days. However, the trend of increasing sensitivity with age is reversed by 3 to 5 months, and may be due to increased hepatic microsomal metabolism of the chemical or decreased chemical permeability of the brain. The implication of this study is clearly that the youngest of the species may not necessarily be the most susceptible to toxaphene poisoning.

Among several field studies to assess the impact on wildlife from insecticide spraying programs, the most recent was conducted by McEwen and coworkers (1972). Toxaphene was applied to a 71,685 ha tract in New Mexico for control of range caterpillar at the rate of 1.1 kg toxaphene in .87 liter of fuel oil per hectare. As the data in Table 5.42 indicate, toxaphene treatment resulted in a marked decrease in bird numbers in comparison to untreated rangeland in the same area. A number of dead birds were found in the spray area including three horned larks, two meadowlarks, one killdeer, one cowbird, and one mourning dove. Observations made during the first week after toxaphene spraying revealed no significant change in bird numbers or mortality.

Table 5.41. Acute oral LD_{50} in mg/kg to Mallards of various ages (95% confidence limits in parentheses)[a]

Chemical	Age		
	36 ± 3 Hr.	7 ± 1 Days	3 - 5 Mo.
Toxaphene	130 (80.4 - 210)	30.8 (23.3 - 40.6)	70.7[b] (37.6 - 133)

[a] Source: Hudson et al., 1972.
[b] Female.

Table 5.42. Bird census on selected plots sprayed with toxaphene[a]

Spray rate (kg/ha)	No. of counts	No. of days post-spray	Avg. no. of birds	
			Pre-spray	Post-spray
1.1	18	8-14	33.6	15.0[b]

[a] Source: Modified from McEwen et al., 1972.

[b] Differs from pre-spray, $\alpha = 0.01$ and from unsprayed area, $\alpha = 0.05$.

In an earlier study conducted by Eyer and coworkers (1953), controlled field conditions were employed to evaluate the effect of toxaphene on geese in cotton fields. Geese of the White Emden, Toulouse, and White China breeds, age 14 to 16 weeks old, were used in the study. Toxaphene was applied to experimental cotton field plots at rates of two to six pounds per acre, either by dust or spray. Some geese remained in the fields both during and after toxaphene application while others were introduced for three days after the spraying or dusting.

Table 5.43 summarizes the results obtained when geese were exposed to toxaphene either by direct contact from spraying or by eating toxaphene-treated vegetation. Toxaphene was apparently safe in either dust or spray when applied at 2.2 kg per ha. At 4.4 kg per ha, birds of the Emden and Toulouse breeds were killed within three hours of direct contact with toxaphene spray. At 5.5 kg/ha, direct contact with toxaphene spray produced death in the Emden breed only within three hours. Sickness followed by recovery was observed in the White China breed. At 6.6 kg per ha none of the geese were affected. This apparent inconsistency in the results was not explained. Symptoms of poisoning in the geese were generally characteristic of chlorinated hydrocarbon intoxication, involving considerable central nervous system effects. Upon post mortem examination, several distinct and consistent lesions were encountered. First, a generalized cyanosis was observed in all birds. In addition, congestion and edema accompanied by hemorrhages were noted in both the heart and spleen. Several birds also displayed swollen and congested livers.

Table 5.43. Toxicity of toxaphene to geese used to control grass and weeds in cotton fields[a]

Treatment	Direct contact	Geese introduced immediately after treatment	Geese introduced 24 hours after treatment	Geese introduced 48 hours after treatment	Geese introduced 72 hours after treatment
Toxaphene, 2.2 kg/ha					
Dust	No[b]	No	No	No	No
Spray	No	No	No	No	No
Toxaphene, 3.3 kg/ha					
Spray	Sickness[c]	No	No	No	No
Toxaphene, 4.4 kg/ha					
Dust	No	No	No	No	No
Spray	Sickness and death	No	No	No	No
Toxaphene, 5.5 kg/ha	Sickness and death	No	No	No	No
Spray					
Toxaphene, 6.6 kg/ha					
Spray	No	No	No	No	No

[a] Source: Modified from Eyer et al., 1953.

[b] No apparent effects.

[c] Birds showing signs of sickness later recovered.

5.4.2.2.2 Chronic -- Data concerning the adverse effects of long-term toxaphene exposures to wildlife are quite limited. Keith (1965) reported the results from a three month toxaphene feeding study using groups of White Pelicans. Groups of five birds each were fed with sardines which had been injected with toxaphene in corn oil to achieve concentrations of either 10 or 50 ppm. Symptoms of poisoning occurred only in the group treated at 50 ppm; bird deaths were recorded at days 29, 32, 34, 43, and 48 of treatment. Upon autopsy, dead birds were noted to be practically devoid of subcutaneous and mesentery fat deposits. Deaths also occurred among control birds. However, these animals generally lived longer and contained greater fat deposits than toxaphene-treated pelicans. A comparison of toxaphene and DDT with respect to toxicity to pelicans revealed that the former insecticide is a more potent poison.

Genelly and Rudd (1956 a) conducted a detailed study on the chronic toxicity of toxaphene to ring-necked pheasants. Four groups of ten female birds were fed toxaphene in their diet for up to three months at levels of 25, 100, 200, and 300 ppm. No mortality resulted in any of the treatment groups. A transitory depression of body weight was noted during the first month of the study, but was attributed to decreased feed consumption. Histological analysis of livers from treated birds demonstrated varying degrees of vacuolation. No other chronic effects resulting from toxaphene treatment were encountered.

Linduska and Springer (1951) found bobwhite quail to be adversely affected by toxaphene at dietary levels of 500 ppm and greater. When incorporated in the diet at 1000 ppm, toxaphene produced 100 percent mortality in 13 days. At a level of 500 ppm in the diet, a 75 percent mortality was achieved in 25 days.

5.4.2.2.3 Other effects -- Several parameters relevant to bird reproduction have been measured in response to toxaphene exposure. Genelly and Rudd (1956 b) determined the effect of toxaphene exposure on egg production, fertility, hatchability, and survival of young in ring-necked pheasants. Their results, as summarized in Table 5.44, show that significant changes were noted in egg laying rate and egg hatchability when hens were fed toxaphene in the diet at 300 ppm. In addition, survival of young birds was significantly depressed by about five percent during the first two weeks following hatching. An evaluation of overall reproductive success obtained by considering the net effect of several parameters revealed the adverse effect of toxaphene treatment (Figure 5.10). A recent report (Bush et al., 1977) indicated that dietary toxaphene levels of 0.5, 5.0, 50, and 100 ppm given to chicks from one day of age through maturity did not significantly alter egg production, hatchability, or fertility.

Dunachie and Fletcher (1969) investigated the effect of toxaphene on the hatching of eggs from White Leghorn hens. Each experiment consisted of four batches of 25 eggs each with one batch serving as a control group. Each egg was injected with toxaphene dissolved either in corn oil or acetone. Controls were injected with solvent only. Their results, depicted in Table 5.45, show some unexplained inconsistencies when using acetone solutions and do not establish a definite dose-response relationship.

Smith and coworkers (1970) examined the effects of toxaphene on egg hatchability. Hens' eggs were injected with the chemical in corn oil directly into the yolk after a seven day incubation period. As shown in Table 5.46, doses of toxaphene up to 1.5 mg per egg did not significantly reduce hatchability.

Eggshell thinning caused by pesticide exposure has been partly responsible for observed reproductive failures in several species of fish-eating and

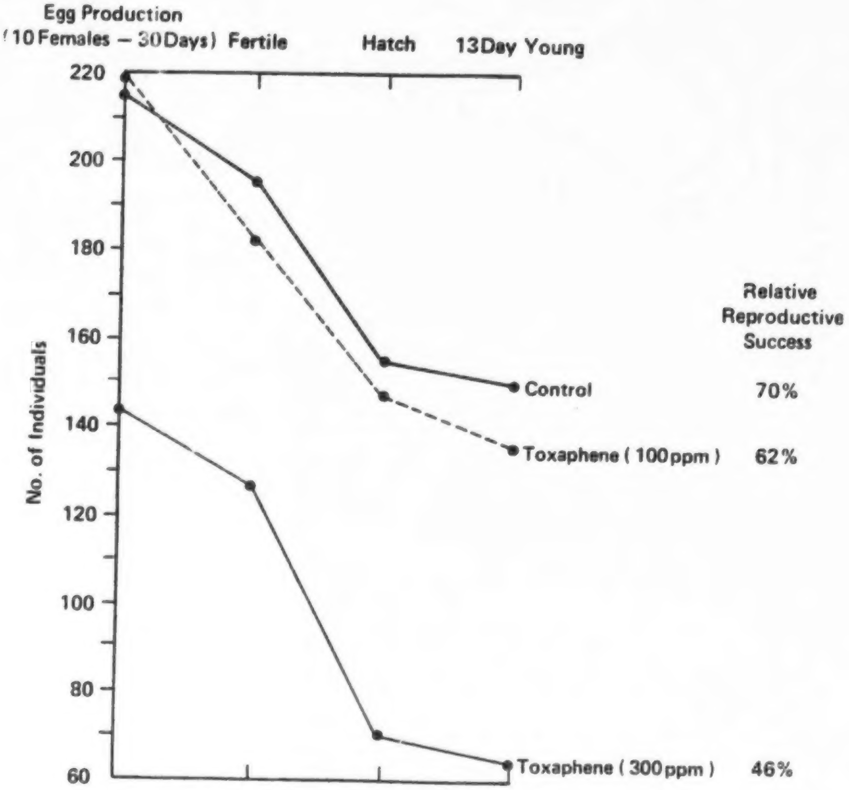


Figure 5.10. Survival curves showing the net effect of toxaphene on ring-necked pheasant reproduction. Source: Modified from Genelly and Rudd, 1956.

Table 5.44. Egg production, fertility, and hatchability[a]

ppm in mash	Hen days	Eggs laid	Laying rate (eggs/♀/day)	Eggs incubated	Eggs fertile	Fertility (per cent)	Young hatched	Hatch-ability (per cent)
Toxaphene								
100	459	333	.725	251	211	84.1	170	80.6
300	918	436	.475[b]	337	297	88.1	165	55.6[b]
Control								
0	1470	1047	.712	785	717	91.3	570	79.5

[a] Source: Modified from Genelly and Rudd, 1956 b.
[b] Differs significantly from control at 5 per cent level.

Table 5.45. The effect of toxaphene on the hatching of hen's eggs[a,b]

Toxaphene ppm	In acetone solvent	In corn oil solvent
500	23/135 (17)	-
400	29/121 (24)	5/36 (14)
300	18/ 75 (24)	5/32 (15.5)
200	27/104 (26)	13/72 (18)
100	49/100 (49)	-
10	12/ 75 (16)	-

[a] Source: Modified from Dunachie and Fletcher, 1969.

[b] Results expressed as ratio of number of eggs hatching to number treated. Figures in parentheses indicate actual percentage of treated eggs hatching.

Table 5.46. Percent hatchability resulting from injection of toxaphene into the yolk of fertile eggs after seven days' incubation[a]

Toxaphene treatment (mg)	% Hatchability[b]
Control[c]	92
0[d]	70.7
0.07	84.0
0.15	77.3
0.30	69.3
0.60	74.7
0.90	70.7
1.50	76.0

[a] Source: Modified from Smith et al., 1970.

[b] Seventy-five eggs per treatment.

[c] Eggs were drilled, sealed, and incubated with no injection whatever.

[d] Eggs were injected with corn oil only.

raptorial birds. Haegele and Tucker (1974) recently investigated the eggshell-thinning effects of numerous pesticides on coturnix quail. They found that a single oral dose of toxaphene at 10 mg/kg of body weight did not significantly reduce eggshell thickness. No other dose levels were employed in this study, nor were additional bird species investigated.

Several investigators have recently become aware of the adverse effects of pesticides on rumen microbial function in wildlife animal species. Schwartz and Nagy (1974) examined the effect of toxaphene on the in vitro fermentation of dry matter (alfalfa hay) inoculated with rumen fluid from yearling mule deer. The results obtained by using rumen bacteria from both wild and penned deer are presented in Table 5.47. Inhibition of dry matter digestion was significant only at toxaphene levels of 1000 ppm.

Table 5.47. Effect of toxaphene on percent digestion of dry matter *in vitro* by Mule Deer rumen bacteria[a]

Rumen bacteria source	Toxaphene levels, ppm			
	0	10	100	1000
Wild deer	42.1[b]	41.3	40.7	28.5[c]
Penned deer	52.5	52.3	51.4	34.5[c]

[a] Source: Modified from Schwartz and Nagy, 1974.

[b] Numbers are percent digestion of dry matter.

[c] Significantly different from controls at $P < 0.05$.

5.5 DOMESTIC ANIMALS

5.5.1 Metabolism

5.5.1.1 Absorption -- No data are available.

5.5.1.2 Transport and Distribution -- No data are available.

5.5.1.3 Biotransformation -- No data are available.

5.5.1.4 Elimination -- No data are available.

5.5.1.5 Residues -- The use of toxaphene for the control of ectoparasites on domestic animals has led to the appearance of toxaphene residues in both meat and milk. Moreover, the ingestion of pesticide-contaminated forage also provides a route of entry for these chemicals into domestic animal tissues.

Data on toxaphene residues in meat and poultry products have been compiled by the U.S. Department of Agriculture (1978) and are summarized in Table 5.48. Toxaphene is found consistently from year to year in the fat of cattle, although the incidence of contamination is extremely low. During the survey period (1973 to 1978) only six samples were in excess of the tolerance limit (7.0 ppm). Of these six violations, five were in fat samples from cattle, one of which occurred in the first quarter of 1978.

Li and coworkers (1970) conducted extensive residue analyses on milk products obtained from dairy cattle after daily feeding of toxaphene at 15 mg/kg of body weight. Samples were taken in duplicate from a group of two cows and toxaphene content analyzed in the fat extracted from their milk. Toxaphene residues in raw milk and in dairy products derived therefrom are summarized in Table 5.49.

In an earlier study conducted by Zweig and coworkers (1963) 16 dairy cows were fed toxaphene at 0 to 20 ppm in the feed for up to 77 days. Samples of milk were analyzed once or twice weekly to determine the presence of toxaphene (measured as total chloride; limit of sensitivity = 0.02 ppm). At all treat-

Table 5.48. Residues of toxaphene in fat samples of meat and poultry products at slaughter in the United States[a]

	Number of Positive Samples/Total Number of Samples (%)					
Animal	1973	1974	1975	1976	1977	1978[b]
Cattle	9/710 (1.27)	2/1117 (0.18)	3/1733 (0.17)	3/1785 (0.17)	4/880 (0.45)	1/432 (0.23)
Calves	1/84 (1.19)	0/284 (0.0)	0/269 (0.0)	0/327 (0.0)	0/124 (0.0)	0/62 (0.0)
Sheep & Goats	2/289 (0.69)[c]	1/371 (0.27)	0/356 (0.0)	0/250 (0.0)	0/100 (0.0)	0/36 (0.0)
Swine	4/232 (1.72)	2/329 (0.61)	0/324 (0.0)	1/442 (0.23)	0/215 (0.0)	0/179 (0.0)
Chicken	3/530 (0.57)	1/1138 (0.09)	0/777 (0.0)	0/927 (0.0)	1/375 (0.27)	0/191 (0.0)
Turkeys	3/517 (0.58)	0/735 (0.0)	0/554 (0.0)	0/456 (0.0)	0/303 (0.0)	0/64 (0.0)
Ducks & Geese	0/95 (0.0)	0/148 (0.0)	0/246 (0.0)	0/267 (0.0)	0/186 (0.0)	0/39 (0.0)
Rabbits	0/19 (0.0)		0/11 (0.0)	0/65 (0.0)	0/21 (0.0)	0/14 (0.0)
Horses	0/44 (0.0)	3/266 (1.13)	0/261 (0.0)	0/217 (0.0)	0/112 (0.0)	0/20 (0.0)
TOTAL	22/2520 (0.87)	9/4388 (0.21)	3/3971 (0.08)	4/4736 (0.08)	5/3216 (0.22)	1/1037 (0.10)

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[a] U.S. Department of Agriculture, 1978.
[b] first two quarters only
[c] listed as lamb

Table 5.49. Toxaphene content of raw whole milk and products manufactured subsequently[a]

Raw whole milk, mg/kg fat	Product	Toxaphene, mg/kg fat	Storage container	Toxaphene in stored samples, mg/kg fat	
				7 Days	14 Days
21.9	Past. whole milk	21.6	Glass	25.0	28.3
			Paper	22.5	22.8
20.8	Past. whole milk	20.4	Glass	22.8	24.8
			Paper	20.5	22.3
21.9	Past. 30% cream	27.2	Paper	29.2	31.7
	Skim milk	36.0			
20.8	Past. 30% cream	23.2	Paper	25.4	27.8
	Skim milk	31.5			
20.8	Ster. cream	30.0			
	Butter	27.1	Parchment[b]		
	Buttermilk	29.2			
21.9	Ster. cream	—			
	Butter	28.7	Parchment[b]		
	Buttermilk	31.0			
21.9	Curd	24.5			
	Whey	28.4			
	Cheese	26.7	Waxed[b]		
28.3	Curd	27.0			
	Whey	30.3			
	Cheese	29.5	Waxed[b]		
28.7	Cond. milk	25.4	Paper	26.5	28.3
	Ster. milk	24.9	Can[b]		
	Dry whole milk	24.7	Poly. bag[b]		
30.1	Cond. milk	—	Paper	—	
	Ster. milk	25.6	Can[b]		
	Dry whole milk	25.0	Poly. bag[b]		

[a] Source: Li et al., 1970.

[b] Samples were analyzed after storage for 3 and 6 months.

ment levels, toxaphene concentrations in milk reached a plateau after the 28th day (Table 5.50). In addition, a rapid decline in toxaphene residues in milk was observed about four days after the pesticide treatment ceased (Table 5.51).

In a similar study by Claborn and coworkers (1963) toxaphene was incorporated in the diet of 14 Jersey cows at levels of 20, 60, 100, and 140 ppm. Two animals were used as controls and three cows were treated at each of the four dosage levels. Total chloride analysis of milk samples (corrected by subtraction of chloride content in untreated samples) demonstrated the presence of toxaphene residues in the milk at all dosages (Table 5.52). Residue levels reached a plateau after about four weeks of toxaphene treatment, and decreased rapidly upon withdrawal from the contaminated feed. Omental fat samples were collected by Claborn et al. (1963) after eight weeks of toxaphene treatment and analyzed for residue content. Residue levels in animals on the 60, 100, and 140 ppm dietary dosages were 8.4, 14.3, and 24.3 ppm, respectively.

Pesticide residues in the milk of dairy cows fed toxaphene-treated alfalfa hay were demonstrated more than two decades ago (Bateman et al., 1953). At an application rate of 1.1 kg per ha of hay, toxaphene was detected (total chloride method) in the milk of two cows at an average of 2.5 and 2.3 ppm over a 112 day period. Hay treated at 2.2 and 4.4 kg of toxaphene per ha produced residues of 4.3 and 3.9, and 18.2 and 8.3 ppm, respectively, in the milk.

Toxaphene sprayed on domestic animals has also resulted in toxaphene residues in various tissues. Claborn and coworkers (1963) applied toxaphene by spray to three groups of two Jersey cows each. Two groups were sprayed twice at three week intervals with toxaphene in the form of either a 0.5

Table 5. 50. Toxaphene concentration (ppm) in milk from cows fed daily rations containing varying amounts of added toxaphene[a]

Days	ppm Toxaphene added to ration				
	2.5	5	10	15	20
2	0.04	---	---	---	---
5	0	---	---	---	---
7	---	0.08	0.13	0.26	0.16
9	0.05	---	---	---	---
11	0.06	---	---	---	---
14	0.03	0.10	0.14	0.18	0.08
18	0.03	---	---	---	---
21	0.02	0.05	0.06	0.11	0.18
25	0.07	---	---	---	---
28	---[b]	0.10	0.19	0.14	0.24
32	0.07	0.07	0.08	0.14	0.20
35	---	0.20	0.10	0.15	0.22
42	---	0.09	0.05	0.13	0.14
49	---	0.05	0.15	0.34	0.26
56	---	0.03	0.11	0.31	0.1[illegible]
63	---	0.01	0.05	0.08	0.11
70	---	0.06	0.12	0.16	0.19
74	---	0.07	0.10	0.09	0.18
77	---	0.08	0.16	0.16	0.18

[a] Source: Zweig et al., 1963.
[b] The 2.5 ppm level was discontinued after the 32nd day.

Table 5.51. Toxaphene concentration (ppm) in milk from cows during feed-off period following feeding of rations containing varying amounts of added toxaphene[a]

Days after toxaphene feeding was discontinued	ppm Toxaphene added to ration				
	2.5	5	10	15	20
1	0.07	---	---	---	---
4	0.03	0.01	0.07	0.09	0.09
7	---	0.01	0.01	0.05	0.02
8	0	---	---	---	---
11	0	0.01	0.03	0.08[b]	0.06
14	---	0	0	0.02	0.02
18	---	0	0	0	0.02
21	---	0	0	0	0.02
25	---	0	0	0	0.01
27	---	0	0	0	0
30	---	---	---	0	0.01
34	---	---	---	0	0
41	---	---	---	---	0

[a] Source: Zweig et al., 1963.

[b] These results for the 11th day and all subsequent dates exclude results of one cow of this group which had become virtually dry.

Table 5.52. Toxaphene (ppm) in milk from cows fed different levels of toxaphene in the diet[a]

(Each sample consisted of 2 liters from morning milk and 1 liter from afternoon milk)

Dosage, ppm	Cow no.	Weeks fed								Weeks after shift to untreated feed		
		1	2	3	4	5	6	7	8	1	2	3
20	3	0.17	0.24	0.24	0.31	0.29	0.33	0.25	0.21	0.10	0.01	----
	4	0.26	0.31	0.31	0.41	0.24	0.42	0.31	0.25	0.06	0.04	----
	5	0.16	0.24	0.24	0.35	0.35	0.35	0.26	0.24	0.06	0.02	----
	Av.	0.20	0.26	0.26	0.36	0.33	0.37	0.27	0.23	0.07	0.02	----
60	6	0.61	0.65	0.74	0.70	0.67	0.68	0.47	0.44	0.08	0.05	0.04
	7	0.61	0.69	0.87	0.66	0.69	0.77	0.53	0.52	0.14	0.11	0.09
	8	0.47	0.50	0.65	0.67	0.53	0.69	0.48	0.48	0.16	0.13	0.09
	Av.	0.56	0.61	0.75	0.68	0.63	0.71	0.49	0.48	0.13	0.10	0.07
100	9	0.90	0.99	0.92	1.06	0.87	0.96	0.93	0.90	0.11	0.05	0.06
	10	0.87	1.00	1.08	1.19	1.13	1.04	0.97	0.96	0.18	0.16	0.15
	11	0.85	1.05	1.04	1.19	0.92	0.89	0.68	0.88	0.17	0.18	----
	Av.	0.87	1.01	1.01	1.15	0.97	0.96	0.86	0.91	0.15	0.13	0.12
140	12	1.46	1.56	1.68	1.75	1.31	1.39	1.36	1.52	0.19	0.17	0.12
	13	1.13	1.09	1.40	1.45	1.23	1.23	1.53	1.44	0.30	0.22	0.21
	14	1.74	2.36	2.32	2.47	1.96	2.31	2.24	2.51	0.46	0.80	0.26
	Av.	1.44	1.67	1.80	1.89	1.50	1.64	1.71	1.82	0.32	0.40	0.20
Control	1	0.00	0.02	0.00	0.07	0.20	0.17	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.04	0.11	0.14	0.00	0.00	0.00	0.00	0.00
	Av.	0.00	0.01	0.00	0.06	0.16	0.16	0.00	0.00	0.00	0.00	0.00

[a] Source: Claborn et al., 1963.

percent emulsion, or a 0.5 percent suspension of the wettable powder. The third group was treated twice daily for 21 days with one ounce of a 2.0 percent toxaphene spray in kerosene. Milk samples obtained from all sprayed cows contained detectable toxaphene residues (measured as total organic chlorine and corrected for chlorine content in control milk samples). These results are presented in Tables 5.53 and 5.54. In all cases, residue levels rapidly decreased after cessation of toxaphene treatment.

Roberts and Radeleff (1960) demonstrated in hogs that toxaphene was probably not substantially accumulated in fat. Four weeks after the spray application of a 0.5 percent toxaphene emulsion, samples of omental and renal fat from eleven hogs contained no chemical residues (determined by a modification of the total chlorine combustion method). Unfortunately, samples were not taken either during or shortly after the toxaphene treatment period.

Dietary levels of up to 100 ppm fed to beef cattle and sheep for 16 weeks resulted in toxaphene residues in omental fat of 20 and 28 ppm, respectively. After discontinuing exposure, fat residues decreased to 12 to 14 ppm at four weeks and 0.5 and 3.0 ppm at eight weeks (Claborn, 1956; Table 5.55). Sheep and cattle fed 25 ppm toxaphene had fat residues of 8 and 12 ppm, respectively, after 16 weeks of feeding. Claborn (1956) also reported on studies in which 12 beef cattle were sprayed with 0.5 percent toxaphene 12 times at 2-week intervals. Omental fat samples were taken surgically prior to each spray application. Table 5.56 shows that after the 12th spraying fat residues were 11 to 16 ppm and had decreased to 4 to 5 ppm four weeks after the last spraying. No toxaphene was detected in the fat of any animals after the first two sprayings.

Claborn (1956) compared fat storage of several organochlorine insecticides in cattle and sheep. He also reported milk residues after spraying 0.5 percent

Table 5.53. Insecticide (ppm) in milk from cows sprayed twice at 3-week intervals with 0.5% sprays of toxaphene[a]

	Emulsion spray[b]	Suspension spray
Sampling date	Toxaphene	Toxaphene
Before spraying	0	0
Days after first spraying:		
1	0.60	0.64
2	0.61	0.74
3	0.44	0.58
5	0.23	0.26
7	0.16	0.20
14	0.06	0.12
21	0.08	0.12
Days after second spraying:		
1	0.52	0.71
2	0.55	0.81
3	0.41	0.57
5	0.24	0.31
7	0.16	0.18
14	0.10	0.08
21	0.06	0.02

[a] Source: Claborn et al., 1963.

[b] The insecticides were calculated in ppm of milk containing 4% butterfat. The values given are averages from the analysis of two milk samples. Each sample consisted of 10 grams of butterfat. The titration of the control sample from each cow was used as the blank and was substracted from subsequent samples.

Table 5.54. Insecticide (ppm) excreted in milk of dairy cows sprayed twice daily for 21 days with 1 ounce of 2.0% oil solution of toxaphene[a]

Sampling date	Toxaphene[b]
Before spraying	0
Days after spraying started:	
1	0.12
3	0.41
7	0.33
14	0.26
21	0.30
Days after spraying ceased:	
7	0.09
14	0.10
21	0.06

[a] Source: Claborn et al., 1963.

[b] Calculated from organic chlorine determinations for milk containing 4% butterfat. The values given are averages from the analyses of two milk samples of 3 liters each.

Table 5.55. Parts per million of insecticides stored in the fat of cattle and sheep that had known amounts added to their diet

Insecticide	Dosage (ppm)	Animal	After feeding				After feeding ceased				
			4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	20 weeks	32 weeks	36 weeks
Toxaphene	100	Ewe	29	30	--	30	19	0			
			21	19	--	21	9	0			
			19	18	25	19	11	0			
		Wether	20	17	--	17	10	0			
			25	19	--	19	15	0			
			16	24	0	17	10	3			
		Av.	22	21	25	20	12	0.5			
		Steer	25	27	36	37	10	--			
			--	45	43	52	29	9			
			30	34	29	29	24	0			
		Heifer	23	27	25	33	10	3			
			26	35	33	39	15	0			
		Av.	26	34	33	38	14	3			
	25	Ewe	1	1	3	11					
			1	1	3	7					
			0	1	1	11					
		Wether	1	0	9	--					
			2	4	1	4					
			4	4	1	6					
		Av.	2	2	3	8					
		Heifer	2	4	11	16					
			3	4	7	12					
			1	9	9	16					
		Steer	4	4	11	8					
			1	1	12	9					
		Av.	2	4	10	12					

(From Claborn, 1956)

Table 5.56. Parts per million of toxaphene in fat of calves sprayed with 0.5 percent toxaphene[a]

Animal	2 weeks after indicated spraying[b]										After 12th spraying	
	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	4 weeks	6 weeks
Emulsion prepared in laboratory												
Steer	0	--	7	--	7	--	--	10	--	13	--	--
	--	11	--	0	--	--	--	--	--	9	--	--
	--	3	--	0	--	10	--	--	10	--	--	0
	2	--	--	--	--	--	--	--	--	--	--	--
	7	--	17	--	--	8	--	--	12	--	--	5
	--	2	--	4	--	--	7	--	--	--	5	--
Av.	3	5	12	1	7	9	7	10	11	11	5	2
Emulsion from concentrate furnished by Humble Oil and Refining Company												
Steer	2	--	9	--	--	--	--	9	--	13	--	--
	--	3	--	4	--	--	12	--	--	20	--	--
	--	11	--	5	--	9	--	--	6	--	--	2
	0	--	13	--	7	--	--	--	--	--	--	--
	0	--	7	--	--	7	--	--	8	--	--	6
	--	--	--	8	--	--	--	--	--	--	4	--
Av.	1	7	10	6	7	8	12	9	7	16	4	4

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[a] All samples taken from the control animals were negative or within the experimental error of the method, which is ± 4 ppm.

[b] None found in any sample after the first two sprayings.

(From Claborn, 1956)

emulsions and suspensions of toxaphene and strobane. Of the insecticides studied the order of their storage in fat from greatest to least was as follows: aldrin > dieldrin > BHC > DDT > chlordane > lindane > endrin > heptachlor > toxaphene > methoxychlor. Methoxychlor was the only insecticide studied that did not cause some storage in the body fat.

Another study (Ent. Res. Service, ARS, USDA, 1959) involved the grazing of range forage treated with 24 oz. toxaphene/A in 1 gal. diesel oil. The forage was analyzed for toxaphene residues and the following results were obtained (Table 5.57).

Table 5.57. ppm Toxaphene on Forage on Days Indicated After Application*

Days	1	3	7	14	42	84
ppm Toxaphene	8.59	4.62	3.50	3.61	1.66	0.99

*Each value represents a composite of 8 sampling sites.

Five yearling cattle were placed on the pasture at the time of spraying and four were placed seven days later. They were kept on the pasture 103 and 96 days, respectively. Omental fat samples were taken: 1) before being placed on the pasture, 2) when removed from the pasture (103rd and 96th day), and 3) at the time of slaughter (120th day). Table 5.58 shows the fat residues of toxaphene. The important finding in this study was that toxaphene residues did not increase above the tolerance of 7 ppm established for meat.

Schanzel and Chemtai (1974) recently noted in a report on problems related to tick control in Uganda, that high levels of toxaphene were being discovered in milk and milk products in Uganda. The authors cited data which revealed levels of 3 ppm in milk, 15 ppm in cream, 21 ppm in cottage cheese, and 87 ppm in milk fat.

Table 5.58. Residues in beef cattle fed hay from fields sprayed with toxaphene

Dosage oz/A	No. of Animals	Days Exposed	Toxaphene in Fat (ppm) Pre-exposure	End of Exposure	At Slaughter
24	5	103	0.1	3.5	0.5
	4	96	0.2	3.2	0.5

(From Ent. Res. Div., ARS, USDA, 1959)

5.5.2 Effects

5.5.2.1 Physiological or Biochemical -- No data are available.

5.5.2.2 Toxicity

5.5.2.2.1 Acute -- Because toxaphene is registered for use in the control of ectoparasites on livestock, several acute toxicity investigations have been conducted employing domestic animals. For the most part, these studies focused on defining "minimum toxic" and "maximum non-toxic" doses.

Radeleff (1949) described in detail the clinical and pathologic signs associated with 27 cases of experimental toxaphene poisoning in goats, sheep and cattle. Each case was detailed individually. The onset of clinical signs occurred within a few minutes to a few hours following exposure. Although not all clinical signs observed were seen in a single poisoned animal, there was sufficient similarity between animals that one can summarize a consistent syndrome associated with toxaphene poisoning.

An affected animal became at first apprehensive and hypersensitive and, in some cases, belligerent. Soon blepharospasms and fasciculations of the facial and cervical muscles appeared, followed by clonic spasms of the cervical muscles, then those of the forequarters and, finally, those of the hindquarters. The spasms were either continuous leading to rapid death or, more frequently, appeared intermittently at regular or irregular intervals concurrent with the appearance of muscular spasms. There was an increased flow of saliva and accompanying chewing movements producing froth. As the seizure activity increased, animals often became frenzied, lost coordination, walked aimlessly about, moved in circles or jumped imaginary objects. Abnormal posturing such as resting the sternum on the ground while the hindlegs remained in the standing position, or persisting in keeping the head between the forelegs while standing were frequently observed. The clinical signs progressed to clonic-tonic seizures accompanied by periods of paddling movements, nystagmus, grinding of the teeth and groaning or grunting in a comatose attitude.

Marsh and coworkers (1951) reported sublethal doses of 35 to 110 mg/kg for toxaphene fed to 17 heifers and young cows weighing from 500 to 800 pounds. The minimum lethal dose was 144 mg/kg. In earlier work, Radeleff (1949) treated goats and sheep by oral drenching with toxaphene. At 100 mg/kg one of two sheep was killed; at 170 mg/kg one of two goats was killed, whereas two sheep survived the same exposure.

Reports from field observations have indicated that young dairy and beef calves are particularly susceptible to poisoning by toxaphene when applied as a spray or dip. Radeleff and Bushland (1950) sprayed calves, two to six weeks old, with three different formulations of toxaphene at various concentrations. Their results, as summarized in Table 5.59, show that death was produced by treating calves at concentrations as low as one percent. The effects of oral toxaphene administration to three month old calves, adult goats, and adult sheep were also investigated. These results are presented in Table 5.60. In addition, the results obtained by treating adult cattle, goats, and sheep with toxaphene sprays or dips are summarized in Table 5.61. A strict adherence to expected dose-related increases in mortality was not observed with any of the animal species or treatments. The nature of the apparent increased susceptibility of young calves to toxaphene poisoning cannot be explained by these results.

Choudbury and Robinson (1950) reported that successive daily oral doses of toxaphene at 25, 37.5, 50, and 75 mg/kg caused no adverse effects in one goat. However, death occurred after feeding 100 mg/kg on days 9 and 10 and 150 mg/kg on day 11. In another animal, a dose of 100 mg/kg on days 1 and 2 followed by 150 mg/kg on day 3 caused death on day 4.

Table 5.5 9. Effects of toxaphene sprays applied to suckling calves[a]

Concentration of toxaphene	Total animals	Number treatments[b]	Formulation administered[c]	Number of animals	Results
8.0%	3	1	XE-65	1	Died
			KE-65	1	Died
			WP-40	1	Affected
4.0%	3	1	XE-65	1	Affected
			KE-65	1	Unaffected
			WP-40[d]	1	Affected
1.5%	12	2	XE-65	4	2 Affected 2 Unaffected
			KE-65	4	1 Died 2 Affected 1 Unaffected
			WP-40	4	3 Affected 1 Unaffected
1.0%	11	1	XE-65	8	1 Died 7 Unaffected
			WP-40	3	3 Unaffected
0.75%	12	8	XE-65	4	1 Affected 3 Unaffected
			KE-65	4	4 Unaffected
			WP-40	4	4 Unaffected

[a] Source: Radeleff and Bushland, 1950.

[b] When animals were treated more than once, the interval between sprayings was 4 days.

[c] XE-65 = An emulsion concentrate containing toxaphene 65 per cent, xylene 25 per cent, Triton X-100 10 per cent; KE-65 = A commercially formulated emulsion concentrate containing 65 per cent toxaphene dissolved in kerosene; WP-40 = A commercially formulated wettable powder containing 40 per cent toxaphene.

[d] Two consecutive sprayings of 4% concentration.

Table 5.60. Effects of oral administration of toxaphene formulations[a]

Animals treated	Dose toxaphene	Total animals	Formulation administered[b]	Number of animals	Results
Calves (3-month-old)	95 mg/kg	3	XE-65	1	Unaffected
			KE-65	1	Unaffected
			WP-40	1	Unaffected
	50 mg/kg	3	XE-65	1	Died
			KE-65	1	Affected
			WP-40	1	Affected
Goats (Adult)	50 mg/kg	3	XE-65	1	Affected
			KE-65	1	Affected
			WP-40	1	Affected
	100 mg/kg	3	XE-65	1	Affected
			KE-65	1	Affected
			WP-40	1	Unaffected
	170 mg/kg	3	XE-65	1	Died
			KE-65	1	Affected
			WP-40	1	Affected
	250 mg/kg	3	XE-65	1	Died
			KE-65	1	Died
			WP-40	1	Died
Sheep (Adult)	50 mg/kg	3	XE-65	1	Unaffected
			KE-65	1	Unaffected
			WP-40	1	Unaffected
	100 mg/kg	3	XE-65	1	Affected
			KE-65	1	Affected
			WP-40	1	Died

Table 5. 60.(continued)

Animals treated	Dose toxaphene	Total animals	Formulation administered[b]	Number of animals	Results
Sheep (Adult) (cont'd)	170 mg/kg	3	XE-65	1	Affected
			KE-65	1	Affected
			WP-40	1	Affected
	250 mg/kg	3	XE-65	1	Died
			KE-65	1	Affected
			WP-40	1	Died

[a] Source: Radeleff and Bushland, 1950.

[b] XE-65 = An emulsion concentrate containing toxaphene 65 per cent, xylene 25 per cent, Triton X-100 10 percent; KE-65 = A commercially formulated emulsion concentrate containing 65 per cent toxaphene dissolved in kerosene; WP-40 = A commercially formulated wettable powder containing 40 per cent toxaphene.

Table 5.61. Effects of toxaphene sprays or dips applied to adult animals[a]

Animals treated	Concentration of toxaphene	Total animals	Number treatments	Formulation administered[b]	Number of animals	Results
Steers	8.0%	1	1	XE-65	1	Affected
	4.0%	20	1	XE-65	7	1 Affected 6 Unaffected
				KE-65	6	6 Unaffected
				WP-40	7	7 Unaffected
Goats	8.0%	3	1	XE-65	1	Died
				KE-65	1	Died
				WP-40	1	Affected
	4.0%	3	1	XE-65	1	Unaffected
				KE-65	1	Unaffected
				WP-40	1	Unaffected
Sheep	8.0%	3	1	XE-65	1	Affected
				KE-65	1	Died
				WP-40	1	Affected
	4.0%	3	1	XE-65	1	Affected
				KE-65	1	Affected
				WP-40	1	Unaffected

[a] Source: Radeleff and Bushland, 1950.

[b] XE-65 = An emulsion concentrate containing toxaphene 65 per cent, xylene 25 percent, Triton X-100 10 per cent; KE-65 = A commercially formulated emulsion concentrate containing 65 per cent toxaphene dissolved in kerosene; WP-40 = A commercially formulated wettable powder containing 40 per cent toxaphene.

5.5.2.2.2 Chronic -- Little information is available concerning the effects of long-term toxaphene exposures to domestic animals. In early studies, Marsh (1949) fed toxaphene-treated hay (8.8 kg/ha) to 14 yearling steers. Treatment continued for more than four months, with daily toxaphene intake averaging 7.9 mg/kg. Two animals developed transient central nervous system effects. Lambs fed toxaphene-treated hay at 1.1, 2.2, 4.4, and 8.8 kg per ha showed no toxic symptoms.

5.5.2.2.3 Other -- In addition to the data which have been accumulated concerning the mortality produced in domestic animals by toxaphene poisoning, recent work has shown that the pesticide may also affect rumen microbial activity. Kutches and coworkers (1969, 1970) investigated the action of toxaphene on in vitro fermentation using rumen liquor obtained from a sheep. They measured pesticide effects on dry matter disappearance, volatile fatty acid production, and changes in protozoal numbers. Toxaphene caused a statistically significant depression of dry matter disappearance of 20 and 28 percent when added to the rumen liquor at levels of 750 and 1,000 ug/ml. No depression was produced at lower concentrations. The authors noted that among the insecticides tested (DDT, sevin, malathion, toxaphene), toxaphene was the most inhibitory. None of the pesticides tested caused a significant decrease in volatile fatty acid production. However, toxaphene produced the greatest depression of protozoal numbers among the pesticides tested.

5.6 TERRESTRIAL INSECTS AND OTHER TERRESTRIAL INVERTEBRATES

5.6.1 Metabolism

Although toxaphene has been used as an insecticide for over twenty years, little is known about the metabolism of this compound in insects. With the exception of a recent study by Crowder (1976), no information is available on the absorption, transport, distribution, and elimination of toxaphene. The rate of toxaphene penetration after topical application has been determined by Crowder (1976) using the American cockroach, *Periplaneta americana*. After application of 75 micrograms of toxaphene per cockroach, penetration into the hemolymph and other body tissue was rapid over the first 8 hours, appeared to stabilize between 8 and 24 hours, and increased markedly between 24 and 48 hours (Table 5.62). The marked increase in absorption between 24 and 48 hours corresponded with the onset of mortality on these animals. The distribution of toxaphene was determined in another cockroach species, *Leucophaea maderae* (Table 5.63).

Insects showing signs of toxaphene intoxication tended to have much higher levels of toxaphene in the hemolymph and the nerve cord at 120 hours after exposure than did insects showing no sign of intoxication. About five percent of the toxaphene associated with the nerve cord seemed to be tightly bound to nervous tissue and was not removed after 20 minutes of rinsing in insect saline. At the subcellular level, toxaphene binding to nerve tissue was greatest in the nuclei, nerve sheath, and microsomal components (Crowder, 1976).

Based on gas chromatographic analysis of toxaphene residues in nervous tissue, Crowder (1976) found no evidence for biotransformation of toxaphene by the cockroach. However, Abd El-Aziz and coworkers (1966) reported the

Table 5.62. Penetration of Cl-36 labelled toxaphene into the American cockroach after topical application of 75 micrograms[a]

Time, hours	Hemolymph		Carcass[b]		Total	
	Micrograms	Percent of applied dose	Micrograms	Percent of applied dose	Micrograms	Percent of applied dose ± SE
1/2	1.11	1.48	8.84	11.78	8.37	11.16 ± 4.0
1	2.79	3.72	8.00	10.67	10.13	13.51 ± 5.8
2	2.15	2.87	5.47	7.29	6.35	8.46 ± 3.4
4	5.08	6.77	7.82	10.42	15.23	20.31 ± 7.7
8	4.57	6.09	11.75	15.66	23.67	31.56 ± 6.5
24	6.04	8.05	12.97	17.29	25.63	34.17 ± 4.3
48	11.09	14.79	51.56	68.75	62.66	83.54 ± 11.7

[a] Source: Crowder, 1976.
[b] Carcass rinsed in acetone to remove unabsorbed toxaphene.

Table 5.63. The distribution of Cl-36 labelled toxaphene in tissues of Leucophaea maderae[a]

Tissue	Number of insects	Disposition[b]	Hour time	Average (μg/g)
Hemolymph	75	A	<120	193.88
	21	A	120	67.02
	12	S	120	563.50
Nerve cord	126	A	<120	654.00
	27	A	120	184.59
	12	S	120	268.71
Fat body	75	A	<120	263.89
	21	A	120	309.62
	12	S	120	299.03
Alimentary canal	75	A	<120	272.97
	21	A	120	260.72
	6	S	120	305.02

[a] Source: Crowder, 1976.

[b] A = Asymptomatic, animals show no signs of intoxication.
S = Symptomatic, animals show signs of nervous system stimulation.

presence of a toxaphene dehydrochlorinase in the dry acetone powder extracts of a toxaphene-resistant strain of the cotton leafworm, Prodenia litura. Using colorimetric methods for the analysis of toxaphene, these investigators estimated 5 to 50 percent breakdown of toxaphene under a variety of in vitro conditions. Gluathione and diethyl ether were necessary cofactors for the reported breakdown of toxaphene.

5.6.2 Effects

5.6.2.1 Physiological and Biochemical Effects -- The mechanism by which toxaphene adversely affects insects is not known. Most investigators feel that toxaphene, like other cyclodiene insecticides, is a neuroactive agent which acts by altering ion permeability in the neurons, rather than by inhibiting cholinesterase (Matsumura, 1975). Using central nerve cord preparations from the German cockroach, Blatella germanica, incubated in 100 micromolar toxaphene solutions, Wang and Matsumura (1970) distinguished three stages of response. The first stage, which began after twelve minutes, was characterized by frequent (5 to 7/second), short (0.03 to 0.05/second) bursts of spontaneous activity with maximum spike intensity of 70 to 80 microvolts. In the second stage, which began after 17 minutes, spike intensity decreased to 50 to 70 microvolts. Bursts became irregular, less frequent (1 to 4/second), and of longer duration (0.1 to 0.4 seconds). After 25 minutes of incubation, spontaneous bursts became weaker, less frequent, and shorter. The threshold of response for toxaphene was 0.6 micromolar, with an average latent period of 14 minutes. Similar bursts of spontaneous nerve discharge were caused by a variety of the cyclodiene insecticides (Wang and Matsumura, 1970). Spontaneous high amplitude, high frequency nervous discharge has also been demonstrated in nerve cord preparations from the American cockroach, Periplaneta americana, at toxaphene concentrations of 1 to 100 millimolar. At lower concentrations, 0.1 to 0.01 millimolar toxaphene had little effect on nerve cord activity. Preliminary observations on the rate of chloride ion uptake by the nerve cord indicated that changes in chloride ion permeability may be related to changes in spontaneous neural activity (Crowder, 1976).

Although toxaphene is not generally considered to act by acetylcholinesterase inhibition, Litzbarski and Litzbarski (1974) have demonstrated in vitro acetylcholinesterase inhibition in both the housefly and Galleria mellonella, the greater wax moth. During ten-minute incubation periods, the toxaphene concentrations causing 50 percent inhibition were about 500 ppm for the moth and 3400 ppm for the fly.

Toxaphene has also been shown to alter formate metabolism in houseflies (Cline and Pearce, 1963). Flies injected with C-14 labelled formate and then treated topically with 3 micrograms toxaphene per fly demonstrated a 5-fold increase in labelled uric acid, but a 2.5-fold decrease in labelled proline after three hours. This effect was also seen with other chlorinated insecticides but not with organophosphate or carbamate insecticides. Because proline is metabolized to gamma-aminobutyric acid which functions in the transmission of nerve impulses, Cline and Pearce (1963) suggested that proline depletion accompanied by a presumed increase in gamma-aminobutyric acid may be involved in the neurotoxic effects of the chlorinated hydrocarbons.

Moore and Taft (1964) were not able to demonstrate in vitro succinic dehydrogenase inhibition by toxaphene in boll weevil homogenates.

5.6.2.2 Toxicity

5.6.2.2.1 Acute Toxicity to Insects

5.6.2.2.1.1 Acute Toxicity of Technical Toxaphene to Insects -- Information on the acute toxicity of toxaphene to various insects in topical application is summarized in Table 5.64. Based on the rather limited number of LD50 estimates given in units of microgram toxaphene per gram insect, the coleopteran, dipteran, and hemipteran pest species, along with the largely beneficial hymenopteran species seem to be susceptible to toxaphene over similar dose ranges, about 2-80 micrograms/gram. In contrast, the lepidopteran species, most of which are economically significant cotton pests, and the coleopteran lady beetles, seem to be much less susceptible to toxaphene with LC50 values commonly over 1000 micrograms/gram. However, the significance of this generalization is difficult to assess. Besides the normal problems in comparing toxicity estimates by different investigators, the development of toxaphene-resistant insect strains may have had a pronounced effect on these toxicity estimates. In an attempt to minimize this effect so that more valid interspecies comparisons can be made, only the lowest LD50 values from studies measuring toxicity in more than one population are presented in Table 5.64. Nevertheless, as discussed more fully in Sections 5.6.2.2.2 and 5.6.2.2.4, toxaphene has enjoyed wide use in the protection of cotton crops from lepidopteran pests for over 20 years. During this time, many strains or populations of these insect pests have become resistant to toxaphene. Thus, the higher LD50 values for the lepidopterans may reflect the high selection pressure for toxaphene resistance on this order of insects as a group and/or the use of highly resistant lepidopteran strains collected from areas of intense toxaphene exposure.

Table 5.64. Acute toxicity of toxaphene to various insects on topical application

ORDER Genus species (Common name)	Stage	Observation period in hours	LD50 (95% confidence interval)		Slope of log dose-probit response line	Reference
			micrograms/gram	micrograms/insect		
COLEOPTERA						
Anthonomus grandis (Boll weevil)	Adult	48	5.43			Fye et al., 1957
		48	240			Roussel et al., 1959
		72	12.5			Burkhalter and Arant, 1960
		72	81.1			Bass and Rawson, 1960
		48		1.296 (0.780-1.812)		Brazzel and Ship, 1962
		48		20.388 (17.815-23.453)		Hopkins et al., 1975
Coleomegilla maculata (Lady beetle)	Adult	72	1789	22		Atallah and Newsom, 1966
Hippodamia convergens (Lady beetle)	Adult	48	1069			Burke, 1959
DIPTERA						
Musca domestica (House fly)	Adult	48	70.0			Ohsawa et al., 1975
	Adult	--	33 (30-35)			Landrum et al., 1976
	Adult	--		2.1		Anagnostopoulos et al., 1974
HEMIPTERA						
Lygus hesperus (Alfalfa bug)	Adult	48		0.463	2.58	Leigh and Jackson, 1968

[a] Dose estimation based on average insect weight. See text for details.

Table 5. 64 (continued)

ORDER Genus species (Common name)	Stage	Observation period in hours	LD50 (95% confidence interval)		Slope of log dose-probit response line	Reference
			micrograms/gram	micrograms/insect		
HYMENOPTERA						
Apis mellifera (Honey bee)	Adult	48		19.08		Graves and Mackensen, 1965
	Adult	48		0.144		Torchio, 1973
	Adult	72	14.2			Johansen and Davis, 1972
Bracon mellitor (a parasitic wasp on the boll weevil)	Adult	48		0.035		Adams and Cross, 1967
Bruchophagus roddi (Alfalfa seed chalcid)	Adult	24		0.676 (0.0512-0.0892)	2.45	Bacon and Riley, 1963
Campoletis perdistivctus (a parasitic wasp on the boll weevil)	Adult, male	24		0.1769 (0.1635-0.1913)	3.36	Lingren et al., 1972
	Adult, female	24		0.2633 (0.2505-0.2767)	5.67	
Megachile rotundata (Alfalfa leafcutting bee)	Adult, female	72	2.12			Johansen, 1972
	Adult, female	72		0.216		Torchio, 1973
Nomia melanden (Alkali bee)	Adult, female	48		0.0023		Torchio, 1973
Vespula pensylvanica (Western yellowjacket)	Adult	48	67.0			Johansen and Davis, 1972

Table 5.64 (continued)

ORDER Genus species (Common name)	Stage	Observation period in hours	LD50 (95% confidence interval)		Slope of log dose-probit response line	Reference
			micrograms/gram	micrograms/insect		
LEPIDOPTERA						
Heliothis armigera	Larva	48	[380.0]	13.61		Wilson, 1974
Heliothis puarctigera	Larva	48	[106.0]	3.70		Wilson, 1974
Heliothis virescens (Tobacco budworm)	Larva	48	[3,280]	82.0		Guzman-Varon et al., 1974
	Larva	48	[2,500]	100.0		Adkisson, 1964
	Larva	48	5,020			Wolfenbarger, 1973
Heliothis zea (Bollworm)	Larva	48	320 (180-460)		0.71	Brazzel, 1964
	Larva	48	[794]	32.0		Adkisson, 1964
Pectinophora gossypiella (Pink bollworm)	Adult	48		0.534		Lowry and Berger, 1964
Prodenia li (Egyptian c n leafworm)	Larva, 4th instar	--	1371 (890-2130)	1	2.0	Salama et al., 1966
	Larva, 4th instar	24	1430			Eldefrawi et al., 1964a
Pseudaletia unipuncta (Armyworm)	Larva	144	56.2			Weisman and Decker, 1951

An additional problem in making valid comparisons among the various species is that most of the acute toxicity data is presented in units of micrograms/insect. While such information may be of some use in intraspecies comparisons, the relatively large variation in size among insect species severely limits the utility of this data in interspecies comparisons. This limitation is most apparent in the hymenoptera data on bees compared to the parasitic wasps. In studies giving the average weight of the insects used, microgram/gram units are estimated in Table 5.64. However, because insect weights in a given species vary widely, no attempt has been made to approximate insect weights and convert LD50 units in the other studies.

For the most part, the methodology used in these studies is similar. Acetone is used as the toxaphene vehicle. Appropriate dilutions are applied in 1 to 3 microliter quantities to the dorsal thoracic region. In all cases, appropriate vehicle controls are used. Control mortality usually ranged from 0 to 3 percent. When excessive mortality was noted in the control group (e.g., Johansen and Davis, 1972), the solvent volume was lowered until an acceptable control mortality was obtained. In most studies, the LD50 estimates were corrected for control mortality using Abbott's (1925) formula.

Although 48 hour LD50 estimates are the most common, some studies give mortality estimates based on 24 or 72 hour exposures. The effect of such variation on the LD50 estimates cannot be defined, given the limited information on the speed of action of toxaphene. Using the residue-film method in toxicity studies on the housefly, Sun (1971) estimated LC50's for toxaphene after 3 and 22 hours at 188 micrograms/jar and 22 micrograms/jar, respectively. Taking a ratio of these values as an index of speed of action, Sun (1971) classified toxaphene as relatively slow acting when compared to a number of other organochlorine insecticides, organophosphorus insecticides, or carbamates. In agreement with the observation, Torchio (1973) noted substantial differences in mortality between 24- and 48-hour exposure periods in three species of bees. However, for the honey bee and the alkali bee, no deaths occurred after 48 hours. With the leafcutting bee, 20% of the deaths occurred between 48 and 72 hours. All bees surviving 72 hours also survived up to 96 hours. If this pattern is common to other insects, 48- and 72-hour LC50's should be relatively similar to each other, but substantially lower than 24-hour LC50's. This is consistent with Crowder's (1976) observation of the rate of toxaphene absorption in cockroaches (Section 5.6.1).

Various factors, such as weight, age, developmental state, sex, and diet, have been shown to affect the toxicity of toxaphene to insects. The effects of weight and age on toxaphene toxicity to the larvae of two lepidopterans have been examined by Eldefrawi and coworkers (1964 a) and Weinman and Decker (1951). In both studies, increasing larval weight was associated with decreasing toxaphene toxicity. In the Egyptian cotton leafworm (Prodenia litura) topical LD50's to 3rd, 4th, and 5th instar larvae were 2.64, 1.43, and more than 7.30 milligrams/gram insect, respectively. The corresponding weight ranges for these instars were 10 to 15 mg, 40 to 60 mg, and 180 to 200 mg (Eldefrawi et al., 1964 a). In the armyworm (Pseudaletia unipuncta), Weinman and Decker (1951) found a well-defined trend between topical toxicity and larval weight. In four groups of larvae weighing less than 150 mg/larva, 151 to 250 mg/larva, 251 to 350 mg/larva, and more than 351 mg/larva, the 48-hour LD50's were 33.7, 49.8, 58.2, and 67.0 micrograms/gram, respectively. These investigators did not indicate which larval instars of the armyworm were tested. While both studies suggest that increasing larval size is accompanied by decreasing toxicity of toxaphene, the lack of confidence intervals for the LD50's prevents an evaluation of the significance of these findings. In both studies, the maximum differences in toxicity among the larval groups are only about 2-fold.

The effect of diapause on toxaphene toxicity has been examined in the adult lady beetle, Coleomegilla moculata (Atallah and Newsom, 1966). The beetles were collected and maintained in diapause until the time of testing, with mating used as the criterion for return to activity. The resulting LD50 for the diapausing beetle (44.2 micrograms/beetle or 3619 micrograms/gram) was about twice that of the active beetle (Table 5.64). These differences were significant at the 0.05 level. As illustrated in Figure 5.11, the slopes of the log dose-probit response lines for both stages are parallel, indicating similar heterogenicity and possibly similar modes of action in the two stages. Atallah and Newsom (1966) conclude that the relatively gradual slopes - e.g., high degree of heterogenicity - were indicative of a population currently developing resistance to toxaphene.

Possible differences in the response of males and females to toxaphene has received little attention. With Bracan mellitor, Adams and Cross (1967) noted lower LD50 values (as micrograms/insect) in males than in females. This difference was attributed to the smaller size of the male wasps. Insufficient information is given in their report to determine if both size and sex could have been involved. For another wasp species, Campoletis perdistinctus, males were also found to be more susceptible than females in terms of dose per wasp (Table 5.64). Although no reference was made to size differences (Lingren et al., 1972), male ichneumonid wasps are generally much smaller than females. In a

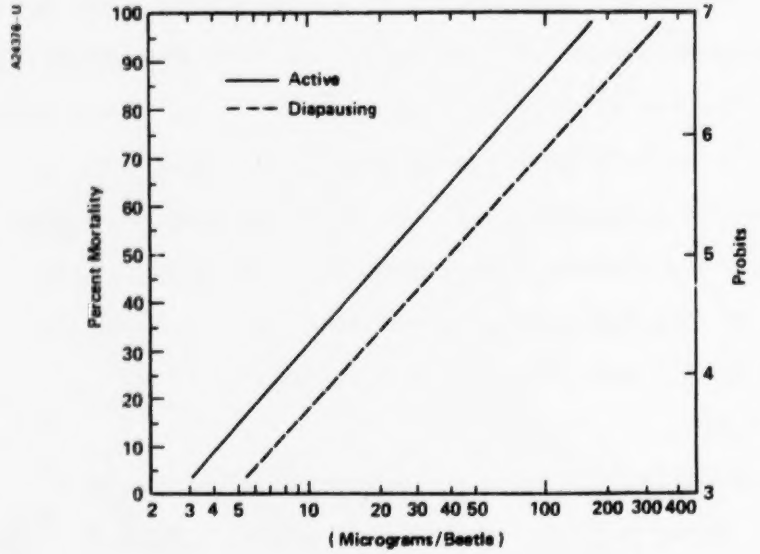

Figure 5.11. Log dose-probit lines for toxaphene treated lady beetles based on 72-hour observations. Source: Atallah and Newsom, 1966.

modified contact toxicity test in which male and female houseflies were exposed to various concentrations of toxaphene in different types of soil samples, male flies were consistently about twice as sensitive to toxaphene based on measurements of LT90's (Qadri, 1972). (Different soil types had no apparent effect on toxicity.)

In the boll weevil, Anthomonus grandis, larval habitat, adult food source, as well as weevil age, have been shown to have significant influence on the topical toxicity of toxaphene (Bass and Rawson, 1960). In their study, larvae were collected either from cotton squares (square-reared) or bolls (boll-reared) and emerging adult weevils were tested at either two or nine days of age. During the test period, adult weevils were fed from only one source, either blooms, bolls, or squares. The results, summarized in Table 5.65, indicated that boll-reared, boll-fed, nine-day old weevils were 61 times more tolerant to toxaphene than square-reared, bloom-fed, two-day old weevils. The single factor contributing most to susceptibility was adult food source. Adults fed on cotton bolls were much less susceptible than those fed on cotton squares, regardless of larval habitat or age. In a field study, Gaines and Mistric (1952) also found that boll-reared larvae were four times more tolerant of toxaphene applications than square-reared larvae.

The oral toxicity of toxaphene has been determined in the 4th instar larva of two lepidopteran species. In both cases, toxaphene was more toxic orally than topically. In the armyworm, Psudaletia unipuncta, the typical LD50 was 56.2 micrograms per gram, while the oral LD50 was 34.1 micrograms per gram (Weinman and Decker, 1951). As in topical applications, smaller larvae seemed more susceptible to toxaphene than larger larvae. In the

Table 5.65. Toxicity of toxaphene to boll weevils having various combinations of larval habitat, age, and adult food[a]

Adult food	Age of weevils (days)	LD50 and fiducial limits in μg/g
		Toxaphene
		Square-reared
Blooms	2	81.1 ± 31.2
Squares	2	140.9 ± 58.4
Squares	9	512.1 ± 194.9
Bolls	2	2,099.0 ± 707.0
Bolls	9	4,385.0 ± 1,544.5
		boll-reared
Squares	2	330.4 ± 102.8
Squares	9	1,067.0 ± 849.8
Bolls	2	4,982.0 ± 153.0
Bolls	9	4,943.0 ± 1,537.5

[a] Source: Modified from Bass and Rawson, 1960.

Egyptian cotton leafworm, Prodenia litura, Salama and coworkers (1966) found a greater proportioned difference between the topical LD50 (1.371 micrograms/gram) and oral LD50 (0.250 micrograms/gram).

Other types of toxicity tests have been conducted on toxaphene. The utility of two of the most frequently used, the immersion method and the dry film method, has been recently evaluated by Hopkins and coworkers (1975). Although such tests are useful in screening for efficiency (Section 5.6.2.2.5), actual dosage data in terms of quantity of toxicant per animal cannot be obtained from such methods. Consequently, such studies are not detailed in this section. They are included, when appropriate, in discussions of synergistic effects (Section 5.6.2.2.3) and efficacy (Section 5.6.2.2.5).

Only one study using injection as the exposure route has been encountered. Crowder (1976) estimated the 48-hour LD50 of toxaphene by injection to the cockroach, Leucophaea moderae, at 2.37 micrograms/gram.

5.6.2.2.1.2 Acute toxicity of toxaphene components to insects -- As discussed previously (Section 2.0), toxaphene is a complex mixture of various components. Three of these components, two in toxicant A and one in toxicant B, have been identified (footnote in Table 5.66). In addition, a few investigators have separated toxaphene into various fractions. The toxicity of some toxaphene fractions and the identified components to houseflies are summarized in Table 5.66. In the seven toxaphene fractions tested by Ohsawa and coworkers (1975), there is no apparent relationship of toxicity to either chlorine content or the presence of toxicants A and B. The least toxic fraction, fraction I, has the same chlorine content as the most toxic fraction, IV. While the two highly toxic fractions (III and IV) both contain toxicant B, fraction V, which contains both toxicant A and B, is somewhat less toxic than technical grade

Table 5.66. Acute topical LD50's of toxaphene and toxaphene fractions to the house fly[a]

	LD50 (micrograms/g)	Toxicity relative to toxaphene[b]	Percent Cl
Technical toxaphene	70	1.0	68.6
Expected values of Fractions I - VII[c]	75	0.93	
Fraction I	316	0.22	68.1
Fraction II	139	0.50	68.3
Fraction III[e]	42	1.67	68.8
Fraction IV[e]	33	2.12	68.1
Fraction V[d,e]	76	0.92	66.8
Fraction VI[d]	101	0.69	64.1
Fraction VII	171	0.41	21.2
Toxicant A[f]	16	4.38	
Toxicant B[g]	32	2.18	

[a] Source: Modified from Khalifa et al., 1974 and Ohsawa et al., 1975.

[b] LD50 of toxaphene÷LD50 of component.

[c] Expected value calculated as harmonic mean, i.e., $(X)_H = N/\Sigma\ (1/Xi)$.

[d] Includes Toxicant B.

[e] Includes Toxicant A.

[f] Toxicant A: Identified as mixture of 2,2,5-*endo*,6-*exo*,8,8,9,10-octachloroborane and 2,5,5-*endo*,6-*exo*,8,9,9,10-octachloroborane (Turner et al., 1975).

[g] Toxicant B: Identified as 2,2,5-*endo*,6-*exo*,8,9,10-heptachloroborane.

toxaphene. An identical pattern is seen in the toxicity of these fractions and components to mice (Table 6.13, Section 6.2.2.1.2). The expected value of the LD50 for fractions I through VII does not indicate any synergistic effect among the toxaphene components.

Landrum and coworkers (1976) also present data on the toxicity of toxaphene fractions and two toxaphene components to houseflies (Table 5.67). Component 1 in this study is the same heptachloroborane designated as toxicant A by Ohsawa and coworkers (1975). Landrum and coworkers (1976), however, found this component to be less toxic relative to toxaphene than did the previous study. Nonetheless, both studies indicate that this heptachloroborane is more toxic than technical toxaphene. The other component identified by Landrum and coworkers (1976), a heptachlorodihydrocamphene, is significantly less toxic than technical toxaphene.

Three additional polychloroborane components of toxaphene, which have been isolated but not yet absolutely identified, were found to be 1.75 to 3.5 times more toxic than toxaphene on topical application to houseflies (Anagnostopoulos et al., 1974).

5.6.2.2.2 Resistance in insects -- Resistance to toxaphene has been demonstrated in several orders of insects including: lepidoptera, coleoptera, diptera, hymenoptera, and hemiptera. Although quantitative estimates of such resistance vary markedly and are sometimes difficult to compare, resistant insect strains are often able to tolerate doses of toxaphene ten times above those causing pronounced mortality in corresponding susceptible strains. For target organisms, such as the lepidopteran, some coleopteran, and hemipteran species, the development of resistance can severely affect the efficacy of toxaphene as a control agent.

Table 5.6 7. The topical toxicity of toxaphene, two column fractions, and two components[a]

Compound or mixture	House fly	
	LD50 (mg/kg)	LD50 relative to toxaphene
Toxaphene	33(30-35)[b]	1.0
Column Fraction 3	40(37-44)	0.83[c]
Column Fraction 7	50(44-55)	0.66[c]
Component 1[d]	31(27-36)	1.10
Component 3[e]	40(36-43)	0.83[c]

[a] Source: Landrum et al., 1976.

[b] The values in parentheses represent 95% confidence limits.

[c] Relative LD50's which differ significantly ($p = 0.05$) from toxaphene.

[d] Same as Toxicant B, Table 5.61.

[e] 2,4,6-*exo*,8,8,9,10-heptachlorodihydrocamphene.

A number of lepidopteran larvae of the noctuidae family have been shown to be resistant to toxaphene or toxaphene:DDT combinations. In Texas populations of *Heliothis virescens* (the tobacco budworm), the development of toxaphene resistance followed the development of DDT resistance. In 1963, this species was resistant to DDT but still highly susceptible to DDT:toxaphene combinations (Brazzel, 1963 and 1964). However, between 1963 and 1965, the LD50 value of a 2:1 toxaphene-DDT mixture increased by a factor of six, going from 0.57 mg/g larva to 3.52 mg/g larva. A similar pattern of resistance was also seen in the closely related pesticide, Strobane (Adkisson, 1967). Over about the same period, a four-fold increase in LD50's was seen for the same species in Louisiana, with the resistant strain having a LC50 of 4.3 to 8.6 micrograms/larva (Graves et al., 1963 and 1967). In a 1970 Mississippi study, which compared the toxicity of a 2:1 toxaphene-DDT mixture to *Heliothis virescens*, resistant strains were found in two of the five sample locations and had values of 450 micrograms/larva and over 1000 micrograms/larva. Comparable values in the strains designated as susceptible ranged from 34 to 52 micrograms/larva. These results indicate that the resistant strains tolerated 9 to 29 times more toxaphene than the susceptible strain (Harris, 1972). However, this study differs in an important respect from the Texas and Louisiana studies in that the susceptibility of the populations was not measured over a period of time. Thus, while the variability in the *Heliothis virescens* population found by Harris (1972) clearly suggests the potential for resistance to develop, the actual development of resistance was not demonstrated. In the same study, no such variability was seen in five populations of *Heliothis yea* (the bollworm). Similarly, in an Australian study, Wilson (1974) has demonstrated a five-fold resistance to toxaphene-DDT in some populations of *Heliothis armigera*, but no resistance in *Heliothis punctigera*.

Resistant populations of the Egyptian cotton leafworm (Prodenia litura), also a noctuid, were found during the early 1960's by Ali and coworkers (1962). The extent of resistance ranged from factors of about 8.5 to 24. However, in a follow-up study, both laboratory-reared progeny of the resistant strains and field populations showed an apparent reversal of toxaphene resistance. This reversal occurred after toxaphene use had been discontinued, with carbaryl and trichlorfon being used as substitutes (Eldefrawi et al., 1964 b).

Two species of coleoptera, Anthonomus grandis (the boll weevil) and Tribolium castaneum (a flour beetle), also have demonstrated resistance to toxaphene. As in studies on the tobacco budworm, an early study in five boll weevil populations in Alabama showed no evidence of developing toxaphene resistance (Burkhalter and Arant, 1960). However, after a couple of years, resistant populations were reported in Georgia and Texas (Brazzel and Ship, 1962; Graves and Roussel, 1962; Tippins and Beckham, 1962). In the Texas study the magnitude of resistance in the field populations varied from negligible to a factor of 142, when compared to a laboratory-reared susceptible strain with a LD50 of 7.6 micrograms/weevil (Brazzel and Ship, 1962). In a Louisiana study, no evidence was found for the development of resistance in field populations of boll weevils. However, Graves and Roussel (1962) did demonstrate a 10- to 12-fold increase in the LD50 of a 2:1 toxaphene-DDT mixture in an experimental population of boll weevils exposed to this mixture for 22 generations. Although continued exposure for 20 additional generations did not result in further resistance, the toxaphene-DDT resistant strain was found to be cross-resistant to three organophosphates and two carbamates.

Some attempt has been made to determine the physiological basis of toxaphene resistance in the boll weevil. In comparing the lipid levels of boll weevils surviving and succumbing to a given toxaphene exposure, Reiser and coworkers (1953) found that surviving animals consistently had about 10 percent more fat per unit body weight. In a study on both laboratory-reared and field collected boll weevils, Moore and coworkers (1970) associated boll weevil survival after toxaphene exposure to higher levels of palmitic and oleic acids and lower levels of stearic, linoleic, and linolenic acids than were found in weevils succumbing to exposure. In a subsequent study, Moore and Taft (1972) have associated survival with significantly higher levels of triglycerides. Further, these investigators found significantly higher levels of triglycerides in untreated controls than in surviving animals, suggesting increased triglyceride use by the animal during toxaphene exposure. No relationship was found between survival and phospholipid levels (Moore and Taft, 1972).

Toxaphene resistance in Tribolium castaneum, another coleoptera, has been demonstrated only in a laboratory-reared, lindane-resistant strain. This lindane-resistant strain demonstrated more than a 9-fold cross-resistance to toxaphene, compared to the lindane-susceptible strain (Bhatia and Pradhan, 1972). No such cross-resistance toxaphene was seen in a DDT-resistant strain of the same species (Bhatia and Pradhan, 1970).

Information on toxaphene resistance in other pest orders is somewhat sketchy. Grayson (1953) reports the development of marginal resistance to toxaphene in the hemipteran Oncopeltus fasciatus (the milkweed bug) after laboratory rearing of 17 generations exposed to sublethal levels of toxaphene. Based on 10-second emersions of the insects in suspensions of toxaphene, the selected strain had a LC50 of 0.345 g/liter and the control strain a LC50 of 0.190 g/liter. In studies of pesticide resistance in the body louse, Pediculus humanus, both

Eddy (1952) and Busvine (1953) indicate that DDT-resistance does not confer cross-resistance to toxaphene. However, DDT-resistant houseflies, *Musca domestica*, have been shown to be cross-resistant to toxaphene and Strobane (Bodenstein and Fales, 1962; Pratt and Babers, 1950). Quantitative estimates of resistance cannot be made based on the data presented in either of these studies.

Toxaphene resistance in beneficial insects also has received relatively little study. Atkins and Anderson (1962) demonstrated that DDT-resistant honey bees, *Apis snellifera*, are remarkably cross-resistant to toxaphene. Toxaphene exposures killing fifty percent of the DDT-susceptible bees in 140 to 163 hours caused no mortality above control levels in DDT-resistant bees over periods greater than 560 hours. In *Bracon mellitor*, a hymenopteran parasite of the boll weevil, no resistance to toxaphene could be demonstrated over a five-generation selection exposure study. However, under the same conditions, resistance did develop to a 1:1 toxaphene-DDT mixture and to DDT (Adams and Cross, 1967). Thus, those studies cited above which indicate a resistance to toxaphene-DDT combinations are not definitive proof of the developed resistance to toxaphene alone.

5.6.2.2.3 *Synergistic effects in insects* -- In the control of agricultural pests, toxaphene has been frequently used in combination with other organochlorine pesticides such as DDT or with organophosphorous insecticides such as methyl parathion. While some field studies indicate that such combinations are more efficacious than toxaphene alone (Section 5.6.2.2.5), relatively few attempts have been made to experimentally demonstrate synergism between toxaphene and these other pesticides. Only about half of these studies provide sufficient data for quantitative estimates of synergistic effects.

A variety of methods are available for the analysis of joint toxic action. Although some entomologists (e.g., Atallah and Newsom, 1966; Wolfenbarger, 1973; Wilson, 1974) seem to prefer the calculation of co-toxicity coefficients proposed by Sun and Johnson (1960) using houseflies, this reviewer will use Finney's (1952) model for the calculation of an expected LC50 based on the joint harmonic mean of the LD50 for mixtures (Table 5.68). This method has enjoyed wide use by toxicologists in the analysis of many pesticide and nonpesticide formulations (e.g., Ohsawa et al., 1975; Smyth et al., 1969). The ratio of expected LD50 to observed LD50 is used as the quantitative estimate of synergism, with ratios of less than 1 indicating a less than additive effect and values greater than 1 indicating a more than additive effect. Using this method to analyze synergistic effects in all possible pairs of 27 chemicals, Smyth and coworkers (1969) noted, based on an analysis of ratio variance, that ratios below 0.40 suggest antagonistic effects, while ratios above 2.70 suggest actual synergisms. Intermediate values (0.40 to 2.7) were attributed to random variation. Although such significance estimates are not necessarily valid for all groups of chemicals, they may serve as useful guides in the analysis of toxaphene synergisms.

Studies on toxaphene synergism from which ratios of expected LD50 values may be calculated are given in Table 5.68. In several studies, an apparent synergism exists between toxaphene and DDT. All four Heliothis species, important cotton pests, show this effect which is also seen in the honey bee and some strains of boll weevil.

In the boll weevil study, some inferences can be drawn concerning the relationship of resistance to synergism. Two strains were used, one sensitive to DDT and toxaphene and the other sensitive to DDT but resistant to toxaphene.

Table 5.68. Synergistic effects of toxaphene with DDT and methyl parathion on topical application

Synergist	Toxa-phene:synergist ratio	Organism	48 hour[a] LD50 toxaphene	48 hour LD50 synergist	Observed LD50 of combination	Expected[b] LD50 of combination	Ratio of expected to observed joint toxicity (E/O)	Reference
DDT	2:1	Anthonmis grandis (Boll weevil)						
		Susceptible	(0.240)	(3.00)	(0.360)	0.265	0.74	Roussel et al., 1959
		Resistant	(6.80)	(2.40)	(0.750)	4.263	5.68	
	2:1	Apis mellifera (Honey bee)	19.08	7.91	4.4	13.10	2.98	Graves and Mackensen, 1965
	1:1	Heliothis armipera						
		Ord strain	22.53	80.71	10.56	35.23	3.33	Wilson, 1974
		Durham strain	13.61	12.24	4.05	12.89	3.18	
	1:1	Heliothis punctizea Ord strain	3.70	0.88	0.30	1.42	4.73	Wilson, 1974
	2:1	Heliothis virescens (Tobacco budworm)	(5.02)	(4.41)	(0.21)	4.85	23.09	Wolfenbarger, 1973
	1:1	Heliothis virescens (Tobacco budworm)	(5.02)	(4.41)	(0.49)	4.70	9.59	Wolfenbarger, 1973
	1:2	Heliothis virescens (Tobacco budworm)	(5.02)	(4.41)	(1.54)	4.61	2.99	Wolfenbarger, 1973
	2:1	Heliothis virescens (Tobacco budworm)	346.0	1.02	0.54	2.03	3.76	Guzman-Varon et al., 1974
	2:1	Heliothis virescens (Tobacco budworm)	78.67	1.26	1.40	2.48	1.77	Guzman-Varon et al., 1974
	2:1	Heliothis virescens (Tobacco budworm)	82.86	17.92	3.34	29.47	8.82	Guzman-Varon et al., 1974

[a] Expected value calculated as harmonic mean, i.e., 1/expected LC50 = Pt/LD50 of toxaphene + Ps/LD50 of synergist where Pt and Ps are proportions of toxaphene and synergist, respectively.

[b] Numbers in parenthesis indicate units of mg/g. All other doses are in units of micrograms/insects.

Table 5.68. (continued)

Synergist	Toxaphene:synergist ratio	Organism	48 hour[a] LD50 toxaphene	48 hour LD50 synergist	Observed LD50 of combination	Expected[b] LD50 of combination	Ratio of expected to observed joint toxicity (E/O)	Reference
DDT	2:1	Heliothis zea (Bollworm)	(0.32)	(2.71)	(0.12)	0.45	3.83	Brazzel, 1964
	[c]	Lygus hesperus						
		1960	0.463	0.894	0.390	0.610	1.56	Leigh and Jackson, 1968
		1961	1.038	0.258	0.252	0.413	1.64	
		1962	0.737	0.877	0.597	0.801	1.34	
	2:1	Pectinophora gossypiella (Pink bollworm) DDT-resistant	0.534	1.164	0.615	0.658	1.06	Lowry and Berger, 1964
Methyl parathion	2:1	Anthonomus grandis (Boll weevil)						
		1973	24.5	0.061	0.194	0.184	0.95	Hopkins et al., 1975
		1974	20.3	0.056	0.123	0.169	1.37	

[a] Expected value calculated as harmonic mean, i.e., 1/expected LC50 = Pt/LD50 of toxaphene + Ps/LD50 of synergist where Pt and Ps are proportions of toxaphene and synergist, respectively.

[b] Numbers in parenthesis indicate units of mg/g. All other doses are in units of micrograms/insects.

[c] Not specified, 1:1 assumed in calculation of predicted LC50.

Marked synergism is noted only in the toxaphene-resistant strain. The increased synergistic effect in this strain was associated with relatively flat and slightly diverging log dose-probit response lines for all applications. In the toxaphene sensitive strain, however, the dose-response line was flat only for the DDT application. The corresponding lines for both toxaphene and toxaphene-DDT applications were much steeper and almost identical to each other (Roussel et al., 1959). This seems to suggest that toxaphene and DDT interacted very weakly, if at all, in the toxaphene-sensitive strain, with most of the lethality attributable directly to the toxaphene. In the study on Heliothis armipera, the Ord strain was more resistant than the Dunham strain to both toxaphene and DDT. However, the levels of synergism in the two strains were about the same (Wilson, 1974). In the tobacco budworm, the most marked synergism is seen in the strain resistant to DDT (Guzman-Varon et al., 1974).

The effect of toxaphene:DDT ratio on synergism has been examined only by Wolfenbarger (1973). Using a strain of Heliothis virescens about equally sensitive to both toxaphene and DDT, this investigator demonstrated that both toxicity and the degree of synergism were directly related to the proportion of toxaphene in the toxaphene-DDT mixture. However, with the boll worm, Heliothis zoa, the highest toxicity was achieved with a 1.1:toxaphene:DDT mixture. Because a LC50 for toxaphene to this species was not given, details of this study are not summarized. However, using a modification of the Sun and Johnson (1960) method and assuming negligible toxaphene toxicity, Wolfenbarger (1973) noted increasing synergistic behavior as the proportion of toxaphene decreased.

The remaining information summarized in Table 5.68 suggests no synergistic effect between toxaphene and DDT in Lygus hesperus or in a DDT-resistant strain of Pectinophora gossypiella. Further, Hopkins and coworkers (1975) were not able to demonstrate a synergistic effect between toxaphene and methyl parathion in the boll weevil. These results are consistent with field studies which indicated that toxaphene does not usually enhance the protective effects of methyl parathion on boll weevil infestations of cotton (Hopkins et al., 1970).

The synergism of dimethyl sulfoxide and toxaphene in boll weevils has been examined by Moore and coworkers (1970). In this study, toxaphene, DDT, and a 2:1 mixture of toxaphene:DDT were applied to a 90 mm disc of filter paper. Boll weevils were left in contact with the treated paper for 48 hours, at which time mortality was recorded. In a parallel series of exposures, 40 percent dimethyl sulfoxide was added to each of the three test preparations. The 48-hour LD50's without dimethyl sulfoxide were 178, 3000, and 1.7 mg insecticide/90 mm filter paper for toxaphene, DDT, and (2:1) toxaphene-DDT, respectively. Using the harmonic mean, these results indicate a synergistic ratio of 154. While supporting the evidence for toxaphene-DDT synergism outlined above, this high synergistic ratio may not be comparable to those described in Table 5.68 because of the differing route of administration. The addition of dimethyl sulfoxide resulted in the following LD50's for toxaphene, DDT, and (2:1) toxaphene-DDT: 141, 1600, and 14.7. While a slight increase in toxicity is apparent for both toxaphene and DDT, the synergistic ratio of the toxaphene-DDT mixture is lowered to about 14.

Although other studies are available in the joint toxicity of toxaphene with some other pesticide, these studies do not provide sufficient data for an analysis of synergism. Many such studies described the development of resistance to toxaphene-DDT combinations (Section 5.6.2.2.2). Others (e.g., Lentz et al., 1974; Rajak and Perti, 1967) are generally screening studies concerned with the development of more efficient pesticide combinations. This aspect of toxaphene synergism is discussed in Section 5.6.2.2.5.

5.6.2.2.4 Reproductive effects in insects -- Remarkably little is known about the long term effects of toxaphene exposure to insects. As reflected in the preceding sections, most of the research generated on toxaphene has been concerned with assessing the usefulness of toxaphene as an insecticide and, secondarily, in determining the immediate effects that such use might have on beneficial insects - e.g., bees and certain parasitic wasps. Consequently, the ability to assess the effects of background levels of toxaphene contamination on insects is limited.

Toxaphene may severely affect the reproductive potential of lady beetles, Coleomegilla maculata (Atallah and Newsom, 1966). After application of single topical doses of toxaphene as low as 10 micrograms per beetle - about 820 micrograms/gram - twenty mated pairs of beetles placed in oviposition cages failed to produce eggs during the remaining life span of the female. Under the same conditions, untreated pairs showed normal egg laying. However, egg production in beetles surviving single doses of a 2:1 toxaphene-DDT mixture at 7.5 micrograms per beetle was not significantly decreased, although the survival potential of the F-1 progeny was reduced by 40%. These results would seem to suggest that DDT blocks the adverse reproductive effects of toxaphene or that the reproductive failure in the toxaphene-only treated group was not due to toxaphene itself.

In a similar study in which milkweed bugs, Oncopeltus fasciatus, were exposed to sublethal levels of toxaphene for 17 bug generations, no significant reduction was seen on the average number of eggs per female (Grayson, 1953).

5.6.2.2.5 Field studies -- Toxaphene, either alone or in combination with other insecticides, has been used as a pest control agent on a number of crops including: alfalfa, cotton, corn, sorghum, soybeans, peanuts, citrus fruits, and various vegetables. An extensive review of these applications giving details on target organisms and recommended application procedures is available from Hercules Incorporated (1972). The efficacy of such application is related to three factors: the suppression of pest species; the effect on beneficial species, such as pollinators or pest predators; and the phototoxic effects of the pesticide application. The phytotoxicity of toxaphene has been discussed in Section 4.0. This section will focus on the effects of toxaphene field applications on pest insects and beneficial insect species.

5.6.2.2.5.1 Pest insects -- Toxaphene is recommended for the control of numerous insect pests (Hercules Incorporated, 1972). Published reports on the effectiveness of toxaphene applications are summarized in Table 5.69. Similar to the estimates of acute toxicity given in Table 5.66, the effects of toxaphene field applications vary considerably from species to species. For instance, toxaphene significantly reduced the *Adelphocoris superbus* infections in alfalfa fields after toxaphene spray applications of .45 - 1.1 kg/ha (Lilly and Hobbs, 1962), while much higher spray applications of up to 22 kg/ha gave inferior control of *Amphimallon majalis* on meadow and pasture sod (Shorey et al., 1958). However, such field studies are particularly difficult to compare to each other. A number of meteorological factors, such as humidity, sunshine, dew, wind, and rain, affect the results of a given field study (Gaines and Mistric, 1952). In most of the studies summarized in this section, sufficient details are not included on these factors to determine their significance in the effectiveness of control. A number of physiological and behavioral characteristics of the insects may also influence the effectiveness of insecticide applications. For example, Gaines and Mistric (1952) have found that toxaphene is more effective

Table 5.69. Efficacy of toxaphene in the control of various insect pests

Pest	Crop	Dose (kg/ha)	Comments	Reference
Aconita dacia (Brown cotton leafworm)	Cotton	2.8	Effective control for up to 6 days	Lloyd and Martin, 1956
Adelphocoris superbus (Superb plant bug)	Alfalfa	0.45 - 1.1	Significantly reduced infestations	Lilly and Hobbs, 1962
Agrotis orthogonia (Pale western cutworm)	Winter wheat	1.1	Failed to give practical control within 7 days	DePew and Harvey, 1957
Agrotis ypsilon (Black cutworm)	Cotton	1.7	Very effective for up to two weeks	Kamel and Shoeb, 1958
Alabama argillacea (Cotton leafworm)	Cotton	2.2	Satisfactory control, 92.8% mortality after 48 hours	Adkisson, 1958
Amphimallon majalis (European chafer)	Meadow and pasture sod	22.0	Inferior control during year of application	Shorey et al., 1958
Blissus leucopterus (Chinch bug)	Sorghum	3.3	Good control for over one week	Randolph and Newton, 1959
Bothynoderes punctiventris (Sugar beet weevil)	Sugar beets	1.0	Up to 100% control	Manolache et al., 1965
Chaetocnema pulicaria (Corn flea beetle)	Sorghum	2.2	75% control for 11 days in 1958 less effective in 1960	Henderson et al., 1962
Coleophora alcyonipennella (Clover case bearers)	White clover	17.0	Effective control	Hoy, 1960

Table 5.69 (continued)

Pest	Crop	Dose (kg/ha)	Comments	Reference
Cotinis nitida (June beetle)	Clover	2.0	Effective for first instar larva, erratic control of second instar	Davich et al., 1957
Eutrombicula alfreddugesi (a chigger)	Woodlands	2.2	Very effective for 4 weeks	Keller and Couck, 1957
Frankliniella sp. (Thrips)	Cotton	0.84	Effective control	Davis et al., 1958
Frankliniella fusca (Thrips)	Peanuts	1.7	90.7% reduction in pest	Dogger, 1956
Frankliniella fusca (Thrips)	Peanuts	1.7	Significant thrip mortality but no increase in crop yield	Morgan et al., 1970
Heliothis zea (Boll worm)	Lima beans	2.46	Adequate control	Stone et al., 1960
Heliothis zea (Boll worm)	Cotton	1.7	58% kill in 48 hours	Nemec, 1972
Kotochalia junodi (Wattle bagworm)	Acacia	2.52	Nearly complete mortality 10 to 14 days after treatment	Ossowski, 1958
Lygus hesperus (Lygus bugs)	Lima beans	3.3	Effective control for up to one month	Stone et al., 1960

Table 5.69.(continued)

Pest	Crop	Dose (kg/ha)	Comments	Reference
Psallus seriatus (Cotton flea hopper)	Cotton	0.84	Effective control	Davis et al., 1958
Pyrausta nubilalis (European corn borer)	Peppers	2.2	Infestation reduced by 50%	Hofmaster et al., 1960
Rhopalosiphum maidis (Corn leaf aphid)	Sorghum	4.9	78% control by 5 days after application	Henderson et al., 1964

in the control of boll weevils when applied early in the growing season. As discussed in Section 5.6.2.2.1, the dietary history of the insect may be important in this effect. The type of toxaphene formulation may also influence the efficacy of toxaphene in a particular species. Dogger (1956) found that 173 kg/ha of toxaphene spray produced a 90.7 percent reduction of a *Frankliniella* species on peanuts. Granulated formations of 10 percent toxaphene, at 224 kg/ha, however, produced only a 64.8 percent reduction in these things. Lastly a variety of different methods are used to assess toxaphene efficacy. While some studies report efficacy in terms of increased crop yield, others use estimates of mortality based on field surveys over periods of a few days to several months. Thus, these field studies, while providing practical information on toxaphene utility, are not readily comparable to each other or to the toxicity estimates given in Section 5.6.2.2.1.

Similar field studies on the efficacy of toxaphene in combination with other insecticides are summarized in Table 5.70. Toxaphene-methyl parathion mixtures are frequently used, and toxaphene-DDT combinations have been commonly used in previous years. As with field studies using only toxaphene, considerable variation is apparent among the different species. The tobacco budworm, *Heliothis virescens*, is only 30 percent controlled by a 1.7 and 2.2 kg/ha application of toxaphene and methyl parathion (Nemec, 1972), while a .56 and 1.1 kg/ha toxaphene-methyl parathion application was effective in the control of the boll worm, *Heliothis zoa* (Turnipseed et al., 1974). A formulation containing a greater amount of toxaphene (1.7 and .56 kg/ha toxaphene-methyl parathion) also produced high (97 percent) mortality in *Heliothis zoa* after 48 hours (Nemec, 1972).

Table 5.70. Efficacy of toxaphene in combination with other insecticides[a]

Pest	Crop	Application (kg/ha)	Comments	Reference
Anthonomus grandis	Cotton	2.2 T + 1.1 MeP	Superior control	Harding 1970
Anthonomus grandis	Cotton	2.2 T + 1.1 DDT	100% kill in 48 hours	Nemec, 1972
Anticarsia gemmatalis	Soybeans	0.56 T + 1.1 MeP	Minimum effective rate	Turnipseed et al., 1974
Frankliniella sp.	Cotton	0.84 T + 0.28 MeP	Effective but no improvement over toxaphene alone; MeP alone had no effect	Davis et al., 1958
Heliothis sp.	Cotton	2.2 T + 1.1 MeP	Significant reduction in boll damage, not much more effective than MeP	Harding, 1972
Heliothis virescens	Cotton	1.7 T + 2.2 MeP	30% kill in 48 hours, significantly more effective than MeP alone but no more effective than toxaphene alone	Nemec, 1972
Heliothis zea	Soybeans	0.56 T + 1.1 MeP	Minimum effective rate	Turnipseed et al., 1974
Heliothis zea	Cotton	2.2 T + 0.84 MeP	Satisfactory control, better than MeP alone	Hopkins et al., 1970
Heliothis zea	Cotton	1.7 T + 0.56 MeP	Almost complete kill within 48 hours, not much better than 0.84 MeP	Nemec, 1972
Heliothis zea	Cotton	1.7 T + 0.56 Ph	Almost complete kill, better than T or Ph alone	Nemec, 1972
Heliothis zea	Cotton	1.7 T + 0.56 G	90% kill in 48 hours, better than T or G alone	Nemec, 1972

[a] KEY: T = toxaphene; MeP = methyl parathion; G = galuron; Ph = phosvel; M = malathion.

Table 5.70 (continued)

Pest	Crop	Application (kg/ha)	Comments	Reference
Heliothis zea	Cotton	1.7 T + 0.56 EPN	87% kill in 48 hours; better than T alone but not as good as 1.1 EPN alone	Nemec, 1972
Heliothis zea	Cotton	2.2 T + 1.1 DDT	30% kill in 48 hours; not as good as toxaphene alone	Nemec, 1972
Lygus hesperus	Cotton	3.3 T + 1.1 M	Effective control	Falcon et al., 1968
Lygus sp.	Lima beans	3.3 T + 1.1 M	Good control for 3 to 4 weeks, better than toxaphene alone	Shorey et al., 1965
Lygus sp.	Lima beans	3.3 T + 1.7 DDT	Less yield than with M combination	Shorey et al., 1965
Plathypenascaba	Soybeans	0.56 T + 1.1 MeP	Minimum effective rate	Turnipseed et al., 1974
Psalius seriatus	Cotton	0.84 T + 0.26 MeP 0.84 T + 0.36 M	Both M and MeP combinations increased cotton yield by 67 kg/ha	Davis et al., 1958
Spodoptera littoralis	Cotton	3.9 T + 1.0 EPN	86% control after 3 days	Kamel and Mitri, 1970

[a] KEY: T = toxaphene; MeP = methyl parathion; G = galuron; Ph = phosvel; M = malathion.

Toxaphene combinations with DDT have given mixed results. In *Spodoptera littoralis*, the Egyptian cotton leafworm, a 3.8, 1.9, and .66 kg/ha combination of toxaphene, DDT, and methyl parathion was almost twice as effective initially as a 5.5 and 1.5 kg/ha combination of toxaphene and methyl parathion (Kamel and Mitri, 1970). Shorey and coworkers (1965), however, found that a toxaphene-DDT mixture was much less effective in the control of several *Lygus* species than a similar mixture of toxaphene malathion. Similarly, in the control of *Heliothis zoa*, a 1.7 and .56 kg/ha mixture of toxaphene and methyl parathion provided a 97 percent kill on cotton in 48 hours, while a 2.2 and 1.1 kg/ha application of toxaphene-DDT caused only 30 percent mortality over the same period (Nemec, 1972). As indicated in Table 5.70, toxaphene combined with organophosphorous insecticides is often effective in the control of many agricultural insect pests. However, as discussed in Section 5.6.2.2.3, quantitative estimates of synergism for these combinations are not available.

5.6.2.2.5.2 Beneficial insects — The limited toxicological data - i.e., LD50 estimates - available on the beneficial insects have been summarized in Table 5.64 and discussed in Section 5.6.2.2.1. These beneficial insects are mostly hymenopterans including two major groups, the bees and the parasitic wasps. In addition, some information was presented on the lady beetle, a coleopteran. However, most of the information on the effects of toxaphene on beneficial insects comes from field studies in which toxaphene was applied and observations made on the effects of treatment on nontarget organisms.

Several insecticide combinations which include toxaphene have been used in the control of pests on alfalfa. Since bees are essential to the pollination of this crop, the control measures must be relatively harmless to bees.

Johansen and Eves (1967) determined the sensitivity of three species of bees to mixtures of toxaphene and three other pesticides (Table 5.71). Both toxaphene-DDT mixtures and toxaphene-oxydemetonmethyl (Meta-Systox-R) caused significant mortality. Toxaphene-systox caused only slight mortality in the alkali bee (Johansen and Eves, 1967). Toxaphene alone was also found to be moderately toxic to the leafcutting bee. Exposure to foliage which had been treated at 3.3 kg/ha toxaphene three hours prior resulted in 35 to 76 percent mortality after 24 hours. When exposure was delayed until 24 and 48 hours after treatment, resulting 24-hour mortalities dropped to 41 to 53 percent and 29 percent, respectively (Johansen et al., 1963). In a similar study, conducted in Egypt, honeybees were exposed to cotton plants which had been sprayed with a 60 percent emulsion of toxaphene at 8.6 liters/ha. Plants held for two or more days before exposure to bees caused less than 10 percent mortality (Wafa et al., 1963). Ibrahim and coworkers (1967), in a related study, assessed the contact toxicity of toxaphene sprays to the honeybee. Toxaphene concentrations of 0.24 to 0.396 percent caused no deaths during six-hour exposures at application rates of 7.4 to 12 liters/ha.

Field studies also indicate that toxaphene is not extremely toxic to bees. Palmer-Jones and coworkers (1958) found no evidence of bee mortality in clover fields treated with toxaphene at 5.5 kg/ha. Only very low bee mortalities (0.77 percent) were seen in fields treated weekly with 4.4 to 17.7 kg/ha toxaphene for eight weeks (Weaver, 1950). However, toxaphene may have a repellant effect on bees. Atkins and coworkers (1970) noticed a decrease in the frequency of bee foraging in fields that had been treated with 4.4 kg/ha toxaphene. Similarly, a 50 percent drop in foraging by honeybees was seen in an alfalfa field sprayed with 3.3 kg/ha toxaphene (Todd and Reed, 1969).

Table 5.71. Alkali bee, leafcutting bee, and honey bee 24-hour percent mortalities from insecticide treatments on alfalfa, Pullman, Washington, 1966[a]

Material	kg/ha	Caged with treated foliage, age of residues											
		Alkali bee				Leafcutting bee					Honey bee		
		3hr	1 day	3 days	5 days	3 hr	1 day	3 days	5 days	7 days	3 hr	1 day	3 days
Toxaphene 3.6 kg E + Demeton (Systox) 0.9 kg E	3.3+ 0.28	10	0	--	--	--	--	--	--	--	--	--	--
Toxaphene 3.6 kg E + Oxydemetonmethyl (Meta-Systox-R) 0.9 kg E	3.3+ 0.28	26[b]	5	--	--	42	18	--	--	--	10	2	--
Toxaphene - DDT 0.9 - 0.6 kg E	3.3 - 1.7	--	40	12	0	--	96[c]	76	24	8	--	22	8

[a] Source: Johansen and Eves, 1967.

[b] Cooler temperature at the time of application caused greater residual action than in Toxaphene-Demeton test.

[c] Daily maximum temperatures were 62 to 75 F during this alfalfa leafcutting bee test.

The reports on the effects of toxaphene on entomophagus insects - i.e., beneficial insects which prey on pest insects - generally indicate that toxaphene adversely affects these animals. Fenton (1959) noted that field treatments with toxaphene (3.3 kg/ha) and several other pesticides caused marked mortality in both beneficial and pest species. However, because of the resulting decrease in entomophagus insects, populations of pest species on treated plots subsequently increased above those in control plots. In cotton and alfalfa fields treated with toxaphene at 2.8 to 3.2 kg/ha, *Orius*, *Geocoris*, *Nabis*, and *Hippodamia* predator species were substantially depressed. Treatments with a 2:1 toxaphene-DDT mixture at 4.9 kg/ha had a somewhat greater effect (van den Bosch et al., 1956). Similar reductions in these insects and in *Sinea diadema* and syrphids were noted in cotton fields treated with 1.5 and 2.9 kg/ha toxaphene-DDT mixtures (Stern et al., 1959). Multiple treatments of cotton fields with toxaphene at 11.1 to 17 kg/ha drastically reduced populations of syrphids, assassin bugs, lady beetles, flower bugs, lace wings, and *Geocoris* species (Gaines, 1955). More recently, Walker and coworkers (1970) have demonstrated that toxaphene treatments of cotton fields initially caused an 80 percent decline in *Hypodamia convergens*, *Orius insidiosus*, and a *Seyminus* species. By three weeks after treatment, populations of these insects were still 60 percent below those in control plots. Shepard and Sterling (1972) have also shown that toxaphene applied to cotton at 1.1 kg/ha reduced by 50 percent the population of various insect predators, including *Orius*, *Seyminus*, *Hippodamia*, *Ceratomeyilla*, *Chrysopa*, *Nabis*, *Podisus*, *Collops*, *Zelus*, and *Geocoris* species.

5.6.2.2.6 *Toxicity to other terrestrial invertebrates* -- A limited amount of information is available on the toxicity of toxaphene to millipedes, spiders, worms, and snails. Of the three species of millipedes tested, only Spinotarsus fiedleri seems susceptible to toxaphene. This species experienced 60 percent mortality by seven days after being fed meal containing 0.2 percent toxaphene. Two other millipede species, Poratophilus pretorianus and Poratophilus robustus, experienced no mortality after identical treatments (Fiedler, 1965). Redmond and Brazzel (1968) tested the toxicity of a 2:1 toxaphene-DDT mixture to the striped lynx spider, Oxyopea salticus. On topical application of 1 microliter drops, the 72-hour LC50's for two spider populations were 0.069 and 0.079 micrograms/milliliter. Sufficient information is not given in this report to convert the LC50 values to LD50's in micrograms toxaphene/gram spider. In a field study, spider populations were initially depressed by 70 percent and remained 50 percent below control levels one month after treatment of a cotton field with toxaphene at 1.1 kg/ha (Walker et al., 1970). As discussed in Section 5.6.2.2.5.2, similar effects were noted on beneficial insect species. Decreases in worm populations have also been seen in worms treated with toxaphene at 6.8 kg/ha (Hopkins and Kirk, 1957) and 1.7 kg/ha (Legg, 1968). Toxaphene has also been shown to cause some mortality in European brown snails at concentrations of 80,000 ppm in bran. No effects, however, were seen at concentrations of 20,000 ppm or 5,000 ppm (Pappas and Carman, 1955).

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6.0 BIOLOGICAL ASPECTS IN HUMANS AND LABORATORY ANIMALS

6.1 CHAPTER SUMMARY

In evaluating the potential hazard of toxaphene to man and other mammals, carcinogenicity should be a major concern. Strobane, a pesticide closely related to toxaphene, has been shown to cause a high rate of liver tumors in mice (Innes et al., 1969). A study by the National Cancer Institute (1979) found that toxpahene, at dietary levels of around 100 ppm given over a one and a half year period, is associated with a high incidence of hepatocellular carcinoma in mice. Rats also evidenced a substantial increase in the incidence of some malignant tumors.

Teratogenicity and other reproductive effects are additional areas of concern. In rats, toxaphene given on days 7 to 16 of gestation resulted in an increased incidence of encephaloceles and resulted in high maternal mortality. Thus, this effect would be of most concern in cases of accidental poisoning of pregnant females. However, even at lower doses to rats and mice - 15 and 25 mg/kg/day - dose related changes were seen in implantation frequency, fetal mortality, and fetal weight gain. In rats only, decreases were seen in sternal and caudal ossification centers (Chernoff and Carver, 1976). Toxaphene has not been shown to be mutagenic using the dominant lethal assay in mice (Epstein et al., 1972).

In other respects, toxaphene is similar to many other chlorinated pesticides in its biological behavior. Toxic amounts of toxaphene may be absorbed across the alimentary tract, skin, and respiratory tract. Toxaphene seems to be distributed throughout body tissues but is stored primarily in the fat (Crowder and Dindal, 1974; Clapp et al., 1971). Toxaphene undergoes dechlorination, which is probably mediated by the microsomal mixed-function oxidase system.

The half-life of toxaphene in rats is about 1 to 3 days and elimination takes place via the urine and feces (Crowder and Dindal, 1974; Ohsawa et al., 1975).

The physiological and biochemical effects of toxaphene resemble those of other chlorinated pesticides. Toxaphene induces the microsomal mixed-function oxidase system and may thus cause a transient decrease in circulating steroid levels (Peakall, 1976). Toxaphene also inhibits ATPases, alters lactate dehydrogenase isozyme patterns, and may affect vitamin A storage (Kuzminskaya and Alekhina, 1976; Phillips and Hatina, 1972).

The acute oral LD50 estimates of toxaphene to various laboratory mammals vary between 25 mg/kg and 270 mg/kg (Table 6.8). As would be expected, the vehicle used has a marked effect on such estimates (Hercules Inc., undated; Lackey, 1949 a). Protein deficiency also increases the sensitivity of rats to acute oral toxaphene intoxication by about a factor of three (Boyd and Taylor, 1971). Although limited documentation on human poisoning is available, the minimum acute oral lethal dose for man has been estimated to be between 30 and 103 mg/kg (Conley, 1952; Pollock, 1958).

The signs of acute oral toxaphene poisoning are similar in all mammals tested. Clonic-tonic convulsions, indicative of central nervous system stimulation, are the signs noted most often. The sequence of events following administration are: a latent period of a few hours, apprehensive behavior, salivation, vomiting, convulsions, and hyperreflexia. Death is sometimes attributed to respiratory failure (Boyd and Taylor, 1971; Haun and Cueto, 1967; Lackey, 1949 a; McGee et al., 1952). Renal tubular damage and fatty degeneration with necrosis of the liver are two predominant pathological signs of acute poisoning (Boyd and Taylor, 1971).

Less detailed data are available on acute poisoning from other routes of entry. The dermal toxicity of toxaphene to laboratory mammals seems to be an order of magnitude below that of oral toxicity (Gaines, 1960 and 1969). While the symptomalogy of acute dermal poisoning is similar to oral intoxication in laboratory animals, dermal exposure to mixtures of toxaphene and lindane may cause acute aplastic anemia in man (Pesticide Episode Response Branch, 1976).

The acute toxicity of some toxaphene components has been estimated (Khalifa et al., 1974; Landrum et al., 1976; Ohsawa et al., 1975; Seiber et al., 1975). Such studies are of considerable interest and may be significant in a future understanding of the mechanism of action of toxaphene poisoning.

The subacute toxicity of toxaphene has not been studied in great detail. Dietary doses of 189 ppm for 12 weeks and 100 to 800 ppm for six months are reported to have no effect in rats (Clapp et al., 1971; Shelanski and Gellhorn, undated). However, 50 ppm for 2 to 9 months has been shown to cause hydropic accumulation in the liver (Ortega et al., 1957). Inhalation exposures to toxaphene dust at 0.012 mg/l for three months causes 50 percent mortality in dogs, but none in rats or guinea pigs (Hercules Inc., undated). In humans, subacute exposure to toxaphene vapor may cause reversible lung damage (Warraki, 1963).

As summarized in the first paragraph of this section, chronic oral exposure to toxaphene is associated with malignant tumors in mice and rats. Toxaphene in the diet at 40 ppm may cause slight liver degeneration in dogs after two years. In monkeys, 10 to 15 ppm in the diet over a two year period is reported to have no effect (Hercules Inc., undated).

6.2 METABOLISM

6.2.1 Absorption

No direct qualitative information is available on the absorption of toxaphene. Crowder and Dindal (1974) have measured the uptake, distribution, and elimination of Cl-36 in rat tissue at various intervals after single oral intubations of 20 mg/kg Cl-36 labelled toxaphene in 0.5 ml peanut oil-gum acacia. However, inconsistencies in toxaphene recovery during the first 24 hours after dosing preclude the use of this data in determining rates of toxaphene absorption (Crowder, 1976). A follow-up study is currently being conducted with mice.

Inferences on the absorption of toxaphene can be made from some of the available toxicity data. Absorption across the alimentary tract, skin, and respiratory tract is indicated by the adverse effects elicited by toxaphene on oral, dermal, and inhalation exposures. Based on toxicity studies detailed in Section 6.2.2, the vehicle used in the administration of toxaphene has a marked influence on lethality which is probably attributable to differences in the extent and/or rate of absorption. In oral exposures, toxaphene has a much lower LD50 when administered in a readily absorbed vehicle - e.g. corn oil or peanut oil - than when given in an indigestible vehicle such as kerosene. Similarly, dermal applications of toxaphene in solution with mineral oil, dimethyl phthalate, or water are much more toxic than similar applications of toxaphene in powder preparations (Lackey, 1949 a and b; Conley, 1952). Documented cases of human poisoning by toxaphene indicate that man may absorb toxic levels on oral, dermal, or inhalation exposures (McGee et al., 1952; Pollock, 1958; Warraki, 1963). When administered or applied in comparable lipophilic solvents, the ratio of oral LD50 to dermal LD50 is about 0.1 (Tables 6.8 and 6.11). This suggests that toxaphene is absorbed more completely and/or more rapidly from the alimentary tract than from the skin. The pronounced variability in time to death after toxaphene ingestion (Section 6.3.2.1.1) indicates marked individual differences in the rate of toxaphene absorption and/or differences in susceptibility to toxaphene intoxication.

6.2.2 Transport and Distribution

Toxaphene seems to distribute readily throughout body tissues soon after dosing. Based upon toxaphene tissue residues in mammals after acute, subacute, and chronic oral exposure, fat tissue seems to be the primary sight of toxaphene storage.

The distribution of Cl-36 in rat tissue at various times after intubation of Cl-36 labelled toxaphene is given in Table 6.1 (Crowder and Dindal, 1974). The values given in this table are average tissue residues for three rats at each period after dosing. The values at day 20 are for three rats redosed with 20 mg/kg on day 9. The quantitative significance of these residue levels at 3 and 6 hours is questionable because of the low recovery of administered dose. Further, the eratic variations of residue levels over the 20 day period are probably attributable to marked variability in toxaphene metabolism among the various experimental animals (Crowder, 1976). Nevertheless, these results do indicate that measurable levels of toxaphene are transported to most body tissues within 3 hours of dosing and are stored in body tissues for at least 9 days. The overall drop in tissue levels between 12 hours and day 1 is consistent with the recovery of large percentages of the administered dose in the feces and urine one day after dosing (Section 6.2.4). Residue levels at

Table 6.1. Uptake of radioactivity in various rat tissues and organs following a single-dose of Cl-36 toxaphene (20 mg/kg)[a]

	% Administered dose[b]									
Tissue / Day	3/24	6/24	12/24	1	2	3	5	7	9	20[c]
Kidney	0.05	0.13	0.43	0.10	0.03	0.03	0.01	0	0.03	0
Muscle	0.93	1.6	5.3	1.3	0	0.65	2.4	0.40	0.14	0.81
Fat	0.14	0.15	0.86	0.57	0.31	0.18	0.18	0.02	3.65	0.03
Testes	0.02	0.08	0.28	0.06	0.04	0.03	0.03	0.02	0.06	0
Brain	0.03	0.06	0.23	0.05	0.04	0	0	0	0.01	0
Blood cells	3.1	0	0	0.06	0.90	2.6	0	0	1.10	1.17
Blood supernatant	0.64	1.20	2.35	1.30	0.60	0.36	0	0.18	0.09	0.06
Liver	0.33	1.10	2.33	0.50	0.31	0	0.01	0	0.48	0
First 2 cm small intestine[d]	0.06	0.34	0.34	0.05	0.01	0	0	0	0.84	0.09
Last 2 cm small intestine[d]	0.10	0.34	0.28	0.13	0.01	0.15	0	0	0	0
Large intestine[d]	0.19	0.60	1.20	0.19	0.08	0.02	0.03	0.04	0	0
Esophagus	0.04	0	0.04	0.03	0.01	0.01	0.02	0	0.03	0
Spleen	0.04	0.06	0.08	0.05	0.02	0	0.03	0.24	0.06	0
Stomach	3.70	18.6	77.20	2.00	0.63	0.61	0.39	0.16	0.12	0
Total	9.37	24.26	90.90	6.39	2.99	4.64	3.10	1.06	6.57	2.16

[a] Source: Modified from Crowder and Dindal, 1974.
[b] Toxaphene in 0.5 ml peanut oil acacia.
[c] Rats redosed with 20 mg/kg on day 9.
[d] Includes contents.

nine days for single-dosed rats and 20 days for redosed rats suggest that a relatively high amount of the retained dose is associated with blood cells. High fat storage, however, is seen only in the single-dosed animals. As discussed above, the quantitative significance of these readings is dubious.

The distribution of C-14 labelled toxaphene and toxicant B (2,2,5-endo, 6-exo,8,9,10-heptachlorobornane) in rats 9 to 14 days after single oral doses is summarized in Table 6.2 (Ohsawa et al., 1975). Fat tissue seems to be the primary site of storage. Elevated levels in the blood, kidney, and liver are suggestive of redistribution from fat via the circulatory system to kidney and liver prior to urinary and fecal elimination (Section 6.2.4). No pronounced differences are apparent between the distribution of technical grade toxaphene and toxicant B.

In twelve week feeding studies of toxaphene to rats at levels of 2.33 to 189 ppm, toxaphene residues - determined by TLC analysis (Section 2.0) - in omental fat, liver, and body composite samples (excluding liver and omental fat) remained below dietary concentrations (Clapp et al., 19[illegible]1). As illustrated in Figure 6.1 for female rats on a diet of 2.33 ppm and 189 ppm toxaphene, toxaphene residues in all tissue samples tended to increase during the twelve week exposure. In male rats, however, liver and omental fat residues peaked after 4 and 8 weeks, respectively. The relation between dietary levels and tissue residues for both male and female rats is illustrated in Figure 6.2. At dietary levels below 21 ppm, all three tissue components contain about the same residue levels. However, as the dietary concentration increases above 21 ppm, tissue levels with respect to composite body samples increase in omental fat but decrease in liver. As in the single dose studies, the pattern of distribution illustrated in Figure 6.2 is indicative of toxaphene storage

Table 6.2. Distribution of C-14 toxaphene and C-14 toxicant B in rat tissue after single intubation[a] (residue in ppm)

	C-14 Toxaphene, 14 days after dosing[c] (8.5 mg/kg)	C-14 Toxaphene, 14 days after dosing[c] (19 mg/kg)	C-14 Toxicant B,[b] 9 days after dosing[c] (2.6 mg/kg)
Fat	0.52	0.78	--
Liver	0.12	0.30	0.12
Kidney	0.17	N.M.[d]	0.09
Blood	0.14	N.M.	0.07
Bone	0.02-0.09	N.M.	<0.03
Brain	0.02-0.09	N.M.	<0.03
Heart	0.02-0.09	N.M.	<0.03
Lung	0.02-0.09	N.M.	<0.03
Muscle	0.02-0.09	N.M.	<0.03
Spleen	0.02-0.09	N.M.	<0.03
Testes	0.02-0.09	N.M.	<0.03

[a] Source: Ohsawa et al., 1975.

[b] 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane (Casida et al., 1974).

[c] Administered in corn oil.

[d] Not measured.

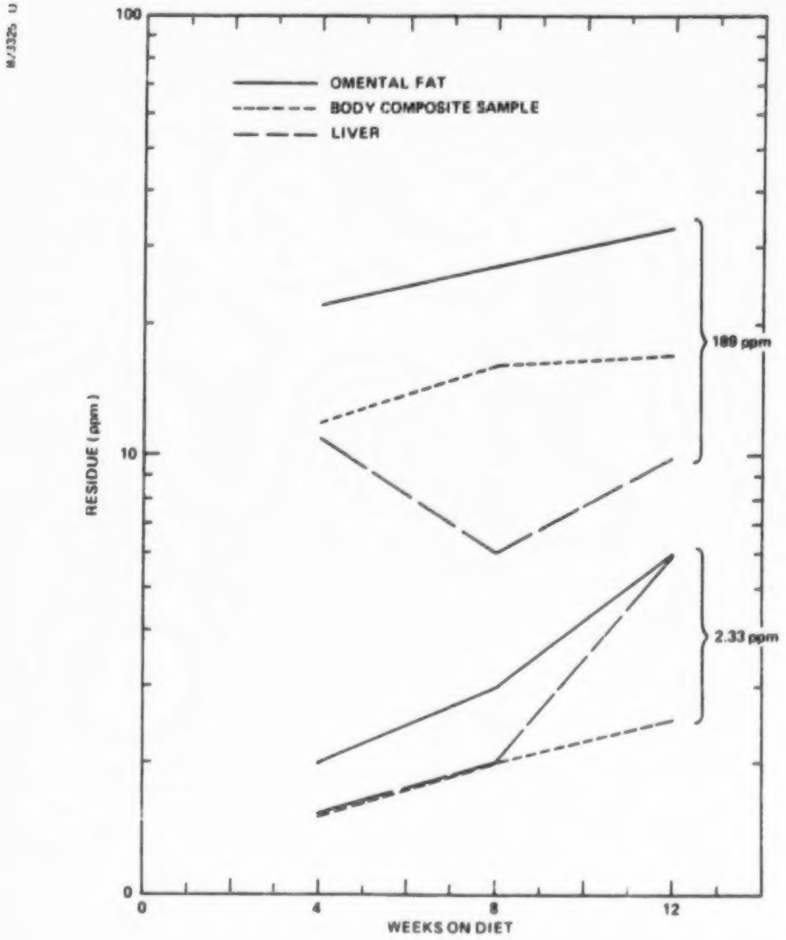

Figure 6.1. Tissue levels in female rats fed toxaphene in the diet at concentrations of 2.33 and 189 ppm.
Source: Modified from Clapp et al., 1971.

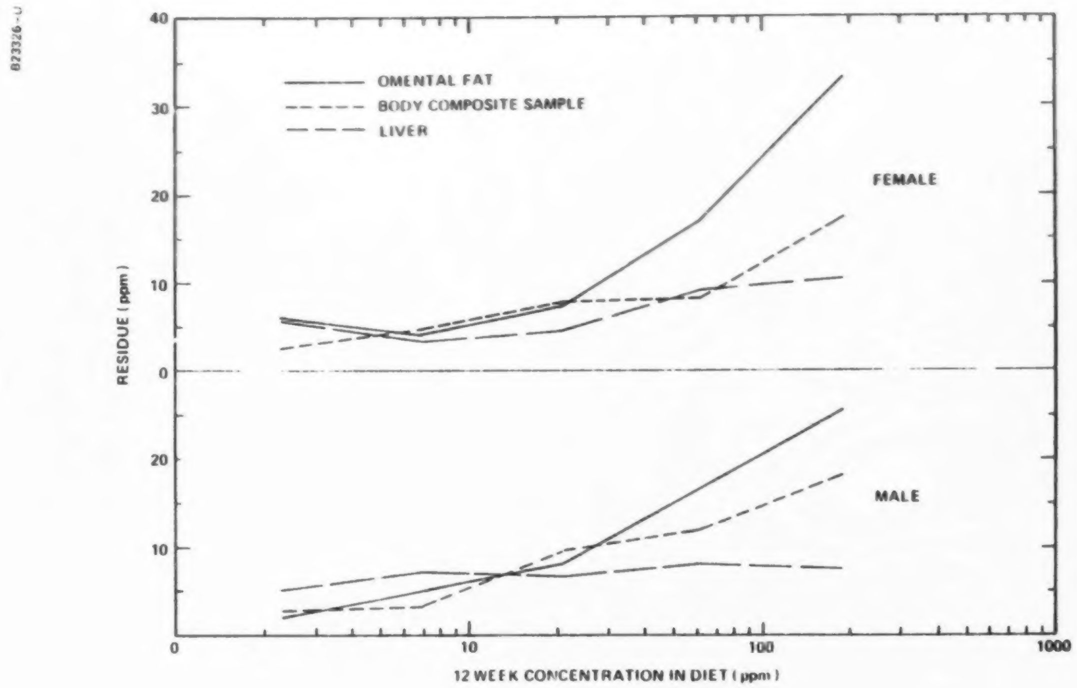

Figure 6.2. The effect of toxaphene concentration in the diet on toxaphene tissue levels in male and female rats after twelve weeks feeding. Source: Clapp et al., 1971.

in fat tissue. The relatively constant liver levels but increasing fat levels suggest that the rate of elimination via liver metabolism may be relatively constant over dietary concentrations of 21 to 189 ppm.

Toxaphene fat storage has been measured in two year feeding studies to rats and dogs. These results are summarized in Table 6.3. The relation of dietary concentration to fat storage in dogs is similar to that seen in the 12 week rat feeding studies illustrated in Figure 6.2. As in the 12 week studies, female rats fed toxaphene for two years store greater amounts of toxaphene in fat than do male rats.

6.2.3 Biotransformation

Dechlorination appears to be the primary metabolic process for toxaphene. In the study by Crowder and Dindal (1974) detailed in Section 6.2.2, about 68 percent of the recovered activity is in the form of ionic chlorine. Of seven Cl-36 labelled toxaphene fractions administered by intubation to rats at doses of 8.4 to 17.2 mg/kg, all were dechlorinated by about 50 percent. Based on the recovery of both C-14 and Cl-36 labelled toxaphene, Ohsawa and coworkers (1975) have concluded that only 3 percent of the original dose of toxaphene (12.7 to 14.2 mg/kg) is excreted unchanged. Only about 2 percent of C-14 labelled toxaphene is eliminated as C-14 labelled carbon dioxide (Ohsawa et al., 1975).

Recently, Khalifa et al. (1976) have demonstrated that toxaphene undergoes reductive dechlorination as well as dehydrochlorination in reduced bovine blood hematin solutions. Similarly, toxaphene underwent about 50 percent dechlorination when incubated with rat liver microsomes and reduced nicotinamide adenine dinucleotide phosphate (NADPH) under anaerobic conditions. Both of these reactions indicate that the hepatic microsomal mixed-function oxidase

Table 6.3. Fat storage of toxaphene in two-year feeding studies of rats and dogs

Animal	Duration (years)	Concn. in diet (ppm)	Concn. in Fat (ppm)	Concentration Factor[a]	Reference
Rat, male	2	400	140	0.35	Lehman, 1952 a
		1600	140	0.088	
Rat, female	2	400	180	0.45	Lehman, 1952 a
		1600	270	0.169	
Dog	0.5	20	4	0.2	Hercules Inc., undated
	1	20	4.2	0.21	
	2	20	5.5	0.275	
	2	10	2.3	0.23	
	2	5	1.7	0.34	

[a] Concentration in fat/concentration in diet.

system (MFOS) may be involved in toxaphene dechlorination. This enzyme system, noted for its broad substrate specificity, utilizes cytochrome P-450 (a protoporphyrin similar to hematin) and requires NADPH. The cytochrome P-450, which serves as the terminal oxidase of this enzyme system, has been shown to bind with a variety of xenobiotics yielding two characteristic spectral patterns, designated type I and type II binding spectra. Toxaphene interacts with the hepatic cytochrome P-450 of rats, mice, and rabbits to yield a type I binding spectra. As detailed by Kulkarni et al. (1975), such spectra usually indicate that the binding compound can serve as a substrate for the cytochrome P-450 enzyme system. Thus, the hepatic microsomal mixed-function oxidase system may play an important role in toxaphene metabolism. Evidence indicating that toxaphene is able to induce this enzyme system is detailed in Section 6.3.1.2.

6.2.4 Elimination

As would be expected from the rapid metabolism and low volatility of toxaphene, urinary and fecal elimination greatly predominate over respiratory elimination. Using Cl-36 labelled toxaphene, Crowder and Dindal (1974) found about twice as much Cl-36 in the feces as in the urine of singly dosed rats. In redosed rats, however, the ratio was approximately equal (Table 6.4).

Ohsawa and coworkers (1975) have noted comparable patterns for Cl-36 and C-14 labelled toxaphene as well as for Cl-36 labelled toxaphene fractions (Table 6.5). In their study, however, the amount of Cl-36 label is consistently twice as great in the urine as in the feces. The C-14 label is distributed more evenly, with a somewhat greater amount consistently found in the feces. Based upon the information summarized in Table 6.5, no significant differences are apparent between elimination patterns of the toxaphene mixture and the fractions or components examined.

Table 6.4. Excretion of radioactivity in urine and feces of rats following a 20 mg/kg dose of Cl-36 toxaphene[a] (% administered dose; N=6)

Single dose				Redose[b]			
Day	Urine	Feces	Total	Day	Urine	Feces	Total
1	1.46	23.95	25.4	10	1.81	6.00	7.8
2	3.20	7.45	10.6	11	3.55	11.60	15.2
3	2.89	1.25	4.1	12	2.26	1.40	3.7
4	2.35	1.10	3.5	13	3.08	1.10	4.2
5	1.82	1.06	2.9	14	3.09	1.20	4.3
6	1.19	1.23	2.4	15	1.77	0.60	2.4
7	1.15	0.69	1.8	16	1.07	0.40	1.5
8	0.54	0.27	0.8	17	0.97	0.40	1.4
				18	1.31	0.20	1.5
				19	0.73	0.00	0.7
				20	0.42	0.00	0.4
Total[c]	15.30	37.30	52.6		20.10	22.90	43.0
Total[d]	29.10%	70.90%	100.0%		46.70%	53.30%	100.0%

[a] Source: Crowder and Dindal, 1974.

[b] The single-dosed animals were redosed with 20 mg/kg on the ninth day.

[c] % Administered dose.

[d] % Recovered dose expressed as 100%.

Table 6.5. Elimination of labelled toxaphene, toxaphene fractions I-VII, and toxicants A and B 14 days after single oral dose[a]

		% Administered dose eliminated in			
Compound	Dose (mg/kg)	Label	Urine	Feces	Expired CO_2
Toxaphene	14.2	C-14	25.2	31.7	1.2
Toxaphene	12.7	Cl-36	49.1	26.9	---
Toxaphene Fraction I	8.4	Cl-36	61.1	29.0	---
Fraction II	14.3	Cl-36	59.6	25.1	---
Fraction III	15.2	Cl-36	47.8	21.5	---
Fraction IV	10.3	Cl-36	62.6	31.4	---
Fraction V	13.5	Cl-36	51.4	23.7	---
Fraction VI	14.9	Cl-36	58.3	20.8	---
Fraction VII	17.1	Cl-36	54.4	18.8	---
Toxicant A	0.84	C-14	28.3	38.4	1.8
Toxicant B[b]	2.6	C-14	26.7	47.8	0.7

[a] Source: Modified from Ohsawa et al., 1975.

[b] Values of toxicant B for nine days after dosing.

The kinetics of toxaphene elimination appear to be first order (Figure 6.3). The half time for toxaphene elimination based on Figure 6.3 varies between 2 and 3 days (Ohsawa et al., 1975). This estimate agrees well with the apparent half-life of toxaphene in Table 6.4 for the redosed animals (t 1/2 approximately 2 days) and the single dosed animals (t 1/2 approximately 1 day).

6.3 EFFECTS

6.3.1 Physiological or Biochemical

6.3.1.1 Normal Physiological or Biochemical Functions -- Toxaphene is an economic poison and is xenobiotic to all known life forms. As such, there can be little reasonable doubt that toxaphene has no normal physiological or biochemical functions.

6.3.1.2 Effects on Normal Physiological or Biochemical Functions -- Like many chlorinated hydrocarbon insecticides such as DDT, toxaphene seems to induce the hepatic microsomal mixed-function oxidase system. However, the inductive potency of toxaphene is generally much less than that of DDT metabolites. A shortening of pentobarbital sleeping time is generally considered a good index of mixed-function oxidase induction. On single oral doses to rats, toxaphene shortens sleeping time only at toxic levels, 50 mg/kg. DDT has a comparable effect at one-tenth this dose (Schwabe and Wendling, 1967). Ghazal (1965) also found that toxaphene, given orally to rats at 100 mg/kg, decreased N-methyl cyclohexenylmethyl barbituate sleeping time by about 20 percent for up to 20 days. Similarly, whole liver homogenates from rats pretreated with toxaphene or DDT in the diet for 1 to 13 weeks showed initial increased activity in three reactions characteristic of microsomal cytochrome P-450: oxidative detoxication of EPN (O-ethyl-O-(4-nitrophenyl)phenylphosphonothioate), O-demethylation of p-nitroanisole, and N-demethylation of aminopyrine

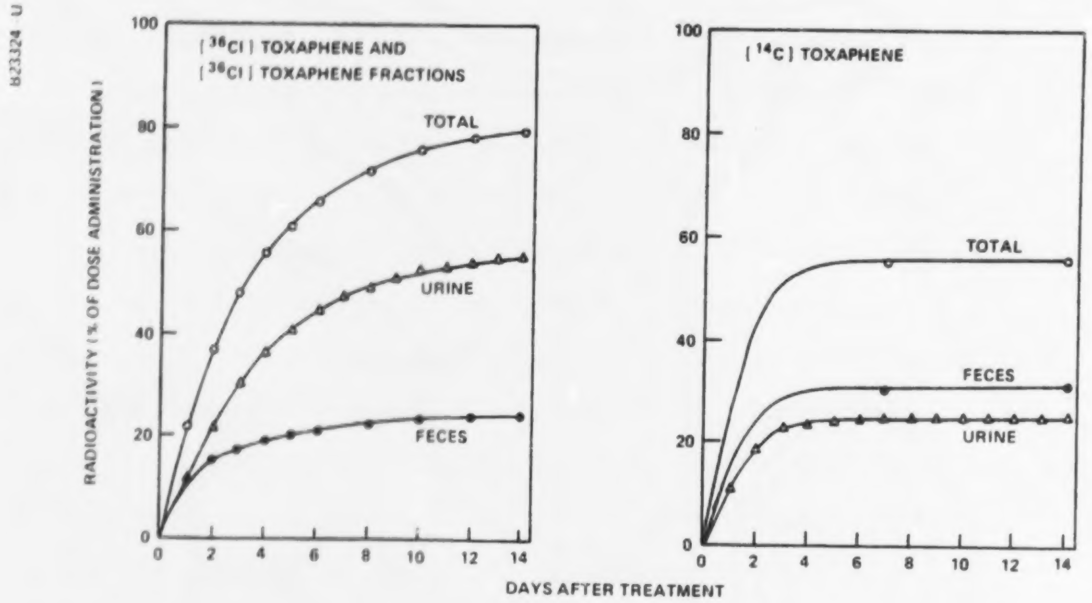

Figure 6.3. Excretion of radioactive products by rats treated orally with [Cl-36] toxaphene, [Cl-36] toxaphene fractions, and [C-14] toxaphene. Source: Ohsawa et al., 1975.

(Kinoshita et al., 1966). In each reaction, DDT had a markedly more pronounced effect than toxaphene (Figure 6.4) and both chemicals generally had a more pronounced effect in male rats (Table 6.6). Toxaphene also stimulates liver microsomal metabolism of estrone and testosterone. Liver microsomal preparations from female rats pretreated with daily intraperitoneal injections of 25 mg/kg toxaphene for seven days showed a 155 percent increase in estrone metabolism. DDT, under the same conditions, resulted in a 213 percent increase (Welch et al., 1971). Similarly, liver microsomal preparations from male rats given a single oral dose of toxaphene at 120 mg/kg evidenced a 484 percent increase in testosterone metabolism by five days after dosing. This increased in vitro metabolism was accompanied by a 19 percent decrease (significant at p less than 0.02) in plasma testosterone. However, by fifteen days after dosing, testosterone levels returned to normal even though microsomal enzyme activity was elevated by 532 percent. Chronic oral exposure to toxaphene - 1.2 mg/kg/day x 6 months - caused comparable increases in microsomal testosterone metabolism but did not lower plasma testosterone levels. Thus, it appears that the initial decrease in circulating testosterone caused by increased liver metabolism is rapidly offset by increased testosterone production (Peakall, 1976). Because toxaphene is known to bind with mammalian cytochrome P-450, giving a typical type I difference spectra (Kulkarni et al., 1975), all of the above metabolic effects may be reasonably attributed to hepatic cytochrome P-450 induction.

Some additional enzyme systems are also affected by toxaphene. At a concentration of 330 micromolar, toxaphene inhibits the in vitro activity of the mitochondrial succinoxidase system of beef heart by 75.9 percent and the

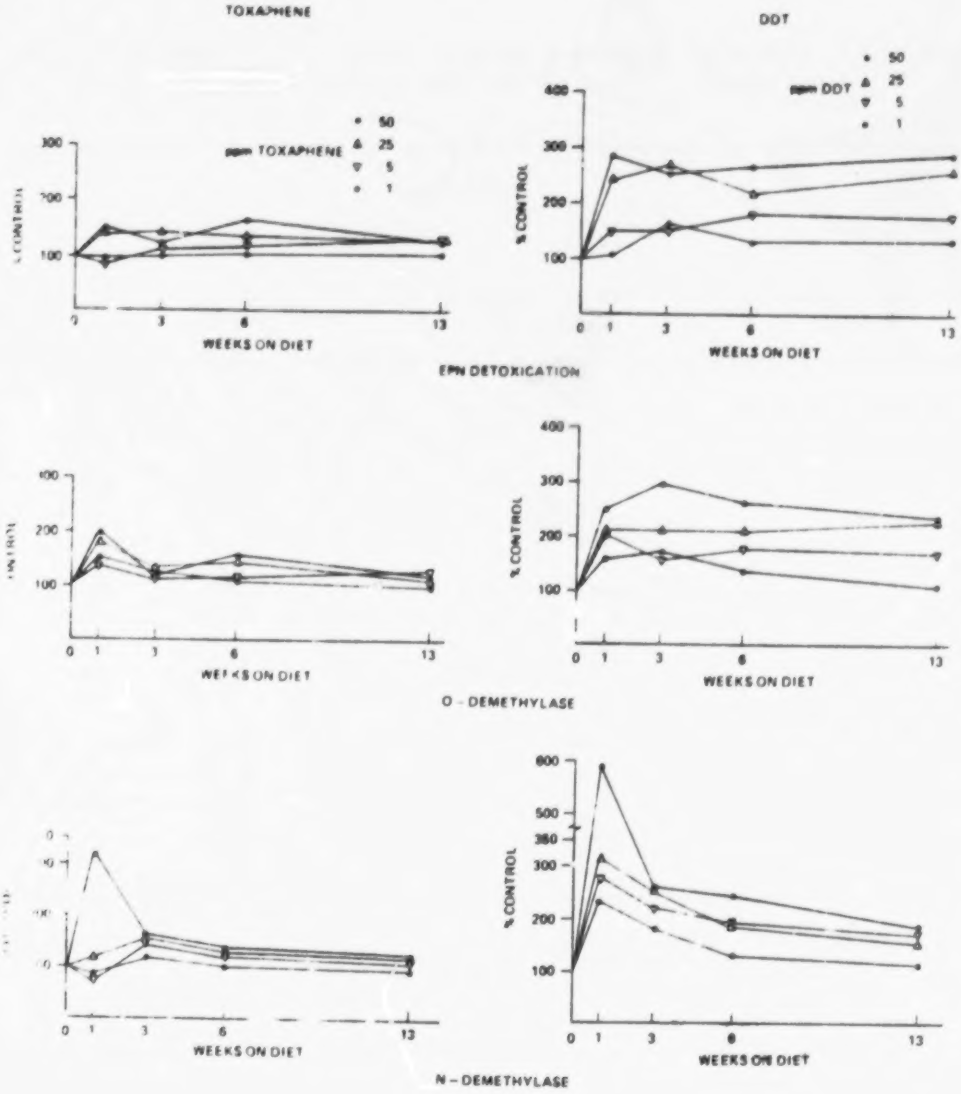

Figure 6.4. Effect of feeding toxaphene and DDT on EPN detoxication, *O*-demethylase activity, and N-demethylase activity of livers of male rats.
Source: Kinoshita et al., 1966.

Table 6.6. Influence of various dietary levels of toxaphene on the activity of microsomal enzymes of the livers of male and female rats[a]

	Activity[b]					
	Dietary level					
Length of feeding (weeks)	Male (5 ppm)	Female (5 ppm)	Male (25 ppm)	Female (25 ppm)	Male (50 ppm)	Female (50 ppm)
EPN Detoxification						
1	90%	114%	145%	146%	150%	135%
3	115%	97%	145%	132%	140%	135%
6	120%	103%	140%	103%	160%	110%
13	125%	90%	140%	110%	150%	130%
O-Demethylase						
1	140%	106%	180%	134%	200%	140%
3	110%	82%	140%	101%	115%	109%
6	120%	113%	150%	112%	155%	95%
13	130%	98%	110%	116%	120%	120%
N-Demethylase						
1	75%	158%	125%	184%	325%	260%
3	140%	127%	155%	161%	160%	224%
6	120%	114%	125%	184%	145%	200%
13	125%	118%	130%	162%	135%	138%

[a] Source: Modified from Kinoshita et al., 1966.
[b] Activity expressed as % of untreated control.

NADH-oxidase system by 96.1 percent (Pardini et al., 1971). At 100 micromolar concentration, toxaphene inhibits by 67 percent the active transport of glucose through isolated mice intestine. However, the mode of action of this effect - inhibition of sugar carrier protein, sodium ion-potassium ion-activated ATPase, or ATP synthesis - has not been determined (Guthrie et al., 1974).

Altered lactate dehydrogenase (LDH) spectra have been demonstrated in the liver and blood of rats given daily oral doses of toxaphene at 1.2 mg/kg for six months or a single oral dose of 120 mg/kg. In the liver, single doses caused a 50 percent decrease in total LDH activity over the 15 day post-exposure observation period. This decreased activity was associated with increases in LDH-1, LDH-2, and LDH-3, a transient decrease in LDH-4, and no significant change in LDH-5. In the blood, single doses caused no significant change in total LDH activity but did cause a transient decrease in LDH-1. Chronic toxaphene exposure decreased total LDH activity in both the blood (30 percent decrease) and liver (45 percent decrease). The only consistent spectral changes in the liver were an increase in LDH-3 and a decrease in LDH-5 after three and six months. In the blood, LDH-1 and LDH-2 activities were depressed and LDH-5 activity elevated after six months. These changes in blood isozyme spectra were thought to be indicative of hypoxia (Kuzminskaya and Alekhina, 1976). Gertig and Nowaczyk (1975) also noted depressed total LDH activity in the liver, kidneys, and serum - 17, 17.5, 20 percent decreases, respectively - of rats given daily oral doses of toxaphene at 35 mg/kg for six months. In kidney LDH isozymes, the only constant change was a moderate (17 percent) but statistically significant drop in LDH-1. These investigators also noted a decrease of about 50 percent in both serum alkaline phosphatase and liver glutamate dehydrogenase after the six month exposure period. Transient changes were seen in serum acid phosphatase (70 percent decrease after one month only), and two liver transaminases (more than 150 percent increase after one and three months). All of the above changes were significant at least by p less than 0.01.

Toxaphene also may have some effect on vitamin A storage. Phillips and Hatina (1972) exposed 28 day old female rats to toxaphene at 100 ppm in the diet for 72 days. After exposure, these rats were mated with untreated males, and liver vitamin A was determined in both dams and progeny at 20 days postpartum. While no effect was seen in the dams, liver vitamin A was depressed by 11.1 percent (statistically significant at p less than 0.01) in the newborn rats. Similar results were also obtained with chlordane, lindane, methoxychlor, and DDT.

The physiological or biochemical effects of toxaphene in man have not received extensive study. In an epidemiological survey of workers exposed to toxaphene and a variety of other pesticides over a period of months, no effects were noted on heme synthesis as determined by delta-aminolevulinic acid and porphobilinogen urinary excretion (Embry et al., 1972).

6.3.2 Toxicity

6.3.2.1 Acute Toxicity -- As discussed in Section 2.0, technical grade toxaphene is a complex mixture of chlorinated alicyclic hydrocarbons. Most of the available acute toxicity information on toxaphene involves the use of this complex mixture. Recently, however, toxaphene has been separated into various fractions and a few of its toxic components have been identified. Information on the toxicity of these components is discussed in a separate section below (Section 6.3.2.1.2).

6.3.2.1.1 Technical toxaphene -- Like many of the chlorinated hydrocarbon pesticides, acute intoxication with toxaphene is characterized by profound central nervous system stimulation. Because of the patterns of toxaphene use and application, oral and dermal exposures are likely to present the greatest hazard to man, and cases of human intoxication by both of these routes have been documented. Consequently, the oral and dermal toxicity of toxaphene to experimental mammals has been studied in some detail.

However, much of the available information consists of only LD50 approximations rather than detailed dose-response data. In that the latter type of information is highly desirable in hazard assessment, all available dose-response data for toxaphene by both oral and dermal administration are presented separately in Table 6.7 and illustrated in Figures 6.5 and 6.6. Details of these studies are discussed in the appropriate sections on oral and dermal toxicity.

6.3.2.1.1.1 Oral toxicity -- The acute oral toxicity of technical grade toxaphene to a variety of laboratory mammals is summarized in Table 6.8. This table includes only toxaphene exposures in which a readily absorbed vehicle is used. A variety of investigators have noted that substitution of such a vehicle with kerosene significantly raises the LD50 estimate of toxaphene. Estimates of this change, as a ratio of LD50 with kerosene to LD50 with vegetable oil, are 1.3 for guinea pigs, 2.0 for rats, and 5.0 for dogs (Hercules Inc., undated). With kerosene as a vehicle, Lackey (1949 a) also noted only sporadic mortality in dogs at toxaphene levels up to eight times above the LD50 with peanut oil. This effect is probably attributable to decreased absorption of toxaphene due to toxaphene affinity for kerosene.

Table 6.7. Estimated dose-response information for toxaphene in oral and dermal applications

	LD1	LD50	LD99	Minimum lethal dose tested	Slope of log dose probit line	Time to death	Reference
Oral							
Rats, male, low protein diet	1.1	80 ± 19[a]	159		--	39 ± 6 hours[a]	Boyd and Taylor, 1971[b]
Rats, male, protein test diet, normal	191	293 ± 31[a]	395		~15	25 ± 13 hours[a]	
Rats, male, standard laboratory chow	139	220 ± 33[a]	300		~15	24 ± 9 hours[a]	
Rats, male	15	90 (67-122)[c]		50	3.0	3 hours-5 days	Gaines, 1960 and 1969[d]
Rats, female	44	88 (70-91)[c]		63	10.0	4 hours-1 day	
Dermal							
Rats, male	72	1075 (717-1613)[c]		800	2	3 hours-4 days	
Rats, female	230	780 (600-1014)[c]		400	4.3	4 hours-5 days	

[a] Mean ± SE.
[b] See Figure 6.6. for graphical presentation of data.
[c] Mean (95% confidence interval).
[d] See Figure 6.7. for graphical presentation of data.

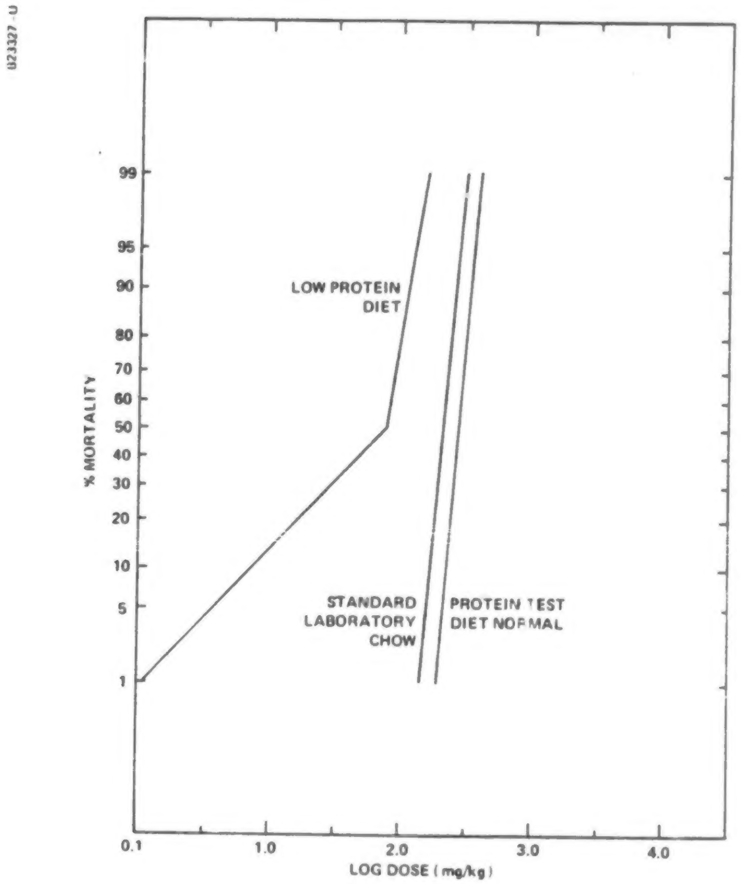

Figure 6.5. Log dose response curves of the acute oral toxicity of toxaphene to rats under different dietary conditions. Source: Boyd et al., 1971.

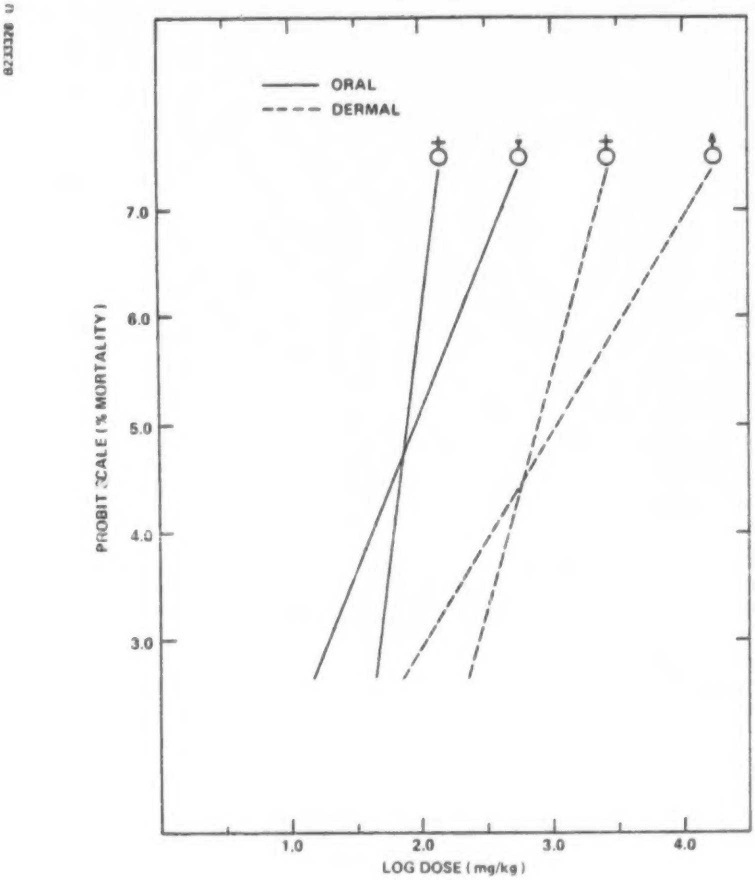

Figure 6.6. Log dose response curves for male and female rats to acute oral and dermal toxaphene intoxication. Source: Gaines, 1960 and 1969.

Table 6.8. Acute oral toxicity of technical toxaphene to laboratory mammals

Organism	Vehicle	LD50 (mg/kg)	Reference
Rats			
Unspecified strain	Unspecified	69	Lehman, 1951
Wistar, male, 3-4 weeks, 50-60 g, fasted	Cottonseed oil	220 ± 33[a]	Boyd and Taylor, 1971
Sherman, male, >90 days, >175 g, fasted	Peanut oil	90(67-122)[b]	Gaines, 1960
Sherman, female, >90 days, >175 g, fasted	Peanut oil	80(70-91)[b]	Gaines, 1960
	Peanut oil	40	Shelanski and Gellhorn, undated
	Peanut oil	90	Hercules Inc., undated
	Corn oil	120-125	Shelanski and Gellhorn, undated
	Corn oil	60	Hercules Inc., undated
Mice	Corn oil	112	Hercules Inc., undated
	Unspecified oil	80	Rico, 1961
Cats	Peanut oil	25-40	Hercules Inc., undated
	Unspecified oil	100	Rico, 1961
Dogs	Peanut oil	∿25	Lackey, 1949 a
	Corn oil	49	Hercules Inc., undated
	Unspecified oil	100	Rico, 1961
Rabbits	Peanut oil	75-100	Hercules Inc., undated
Guinea Pigs	Corn oil	270	Hercules Inc., undated
	Unspecified oil	80	Rico, 1961

[a] ± Standard error.
[b] 95% Confidence interval.

Even when comparable readily absorbed vehicles are used, considerable variation is still evident both within and among the various species cited in Table 6.8. Such variability could be attributable to a number of factors including differences in strain, sex, age, handling, care, and initial health of the experimental animals. Based on the results of Gaines (1960, 1969), sex does not appear to be a significant variable in the LD50 estimate for Sherman rats. However, on both oral and dermal administrations, the dose response curve for female rats has markedly higher slopes than that for male rats (Table 6.7, Figure 6.6). Because confidence limits are not given for these slopes, the significance of this difference cannot be determined. Diet, however, may have a pronounced effect on estimates of toxaphene toxicity. Boyd and Taylor (1971) have shown that rats on a protein deficient diet (3.5 percent casein) for 28 days prior to dosing have LD50 values about three times below those of rats fed on a diet containing normal protein levels (24 to 26 percent casein). Details of these results are summarized in Table 6.6 and Figure 6.5. Similar increases in susceptibility of protein-deficient rats to chemical insult have been demonstrated not only for toxaphene but also for a variety of chlorinated hydrocarbon and organophosphorous insecticides (Boyd and Taylor, 1971). The reasons for the increased sensitivity of protein-deficient animals is not completely understood. However, as indicated in Section 6.2.3, the hepatic microsomal cytochrome P-450 system may contribute to toxaphene detoxication. In that protein deficiency generally depresses cytochrome P-450 concentration and activity (Campbell and Hayes, 1974), one of the factors involved in the increased susceptibility of protein-deficient animals to toxaphene may be a decreased detoxication ability. As illustrated in Figure 6.5, the increased susceptibility of the protein-deficient rats is not uniform throughout the dose-response curve. The disproportionately low LD1 estimate for protein-deficient rats suggests that a subpopulation of this group has an extraordinarily low tolerance to toxaphene poisoning.

Because of the many experimental variables that could influence the LD50 estimates given in Table 6.8 and because confidence limits are not given for most of these estimates, no significant differences in species susceptibility can be identified. The acute oral LD50 estimates for laboratory mammals range from 25 mg/kg to 270 mg/kg. The minimum acute lethal dose for man has been estimated to lie between 30 and 103 mg/kg (Conley, 1952; Pollock, 1958). Based on the estimates of mammalian toxicity discussed above and on cases of accidental ingestion by humans detailed below, this approximation of human lethal dose seems reasonable.

Like most of the chlorinated hydrocarbon insecticides, toxaphene acts as a central nervous system stimulant in acute intoxication. The precise mechanism of this effect, however, is not known. The most consistent clinical sign of acute toxaphene poisoning is clonic-tonic convulsions. In fatal exposures, death is usually attributable to respiratory failure. For the most part, patterns of intoxication are similar in dogs, rats, and humans. Published reports of human cases of toxaphene poisoning by ingestion are summarized in Table 6.9. Initial signs of intoxication usually do not appear until a few hours after ingestion. Rats responded to single oral doses of toxaphene with hypersensitivity, tremors, and convulsions. Symptoms appeared after 1 1/2 hours and lasted for up to 24 hours. Although some delayed deaths were noted up to the sixth day after dosing, most deaths occurred during the first 24 hours (Lehman, 1951). In dogs receiving lethal doses of toxaphene, the

Table 6.9. Case studies of toxaphene poisoning in humans in which ingestion is the primary route of entry[a]

Case No.	1	2	3	4	5	6	7
Subject(s)	Male 2 yr 8 mo	Male, 4 yrs	Male, 1 yr 5 mo	Male, 2 yrs	Female, 20 yrs Female, 16 yrs Female, 12 yrs	Male, adult Male, young Female, adult	Female, 9 mo
Nature of toxaphene	Wax	Emulsion in water	60% in solvents	20% in solution	Residue of spray in food	Residue of spray in food	Powder, 13.8% toxaphene, 7.04% DDT
Dose	Unknown	Unknown	~100 mg/kg[b]	Unknown	9.5-47 mg/kg	Unknown	Unknown
Time to react to onset of symptoms	~7 hours	2 hours	N.S.	N.S.	1.5-4 hours	4 hours	A few hours
Symptoms	Convulsions	Convulsions at 2-5 minute intervals	Convulsions, intermittent	Convulsions, intermittent; mild cerebral excitement; aimless jerking motion and excessive muscular tension of extremities; marked pharyngeal and laryngeal spasms; labored respiration; cyanosis	Nausea; vomiting; convulsions	No nausea; spontaneous vomiting; convulsions; jerking and transitory movements; muscular rigidity; periods of unconsciousness; amnesia(?)	Vomiting; diarrhea; convulsions; hyperreflexia; tachycardia; b.p.140/110; labored respiration; respiratory failure
Outcome	Death	Death	Death	Recovery	Recovery	Recovery	Death
Time to death or recovery	9.5 hours	6 hours	11 hours	12 hours	~12 hours	<1 day(?)	~9 hours

[a] Sources: Cases 1-6, McGee et al., 1952; Case 7, Haun and Cueto, 1967.
[b] See text for dose estimation.

first signs of intoxication include apprehensive behavior, salivation, and vomiting which appear 1 1/2 to 2 hours after dosing (Lackey, 1949 a). In some cases of human poisoning, however, early signs of intoxication may be severe - e.g., Case 6 in Table 6.9. Along with convulsions, hyperreflexia has been noted in dogs (Lackey, 1949 a), rats (Boyd and Taylor, 1971), and humans (Haun and Cueto, 1967). In Case 7 in Table 6.9, hyperreflexia was an outstanding feature in the latter stages of intoxication. While Doroshchuk (1974) indicates that both nembutal and urethane can be used to control spasms and normalize respiration in severely intoxicated rats and cats, Haun and Cueto (1967) found that phenobarbital did not diminish the severe "violent spasm" induced by "very minor stimulation" in Case 7 in Table 6.9. However, except for the consistent presence of convulsions, the symptoms seen in cases of acute human intoxication are somewhat variable. For instance, Case 6 in Table 6.9 is somewhat peculiar in that neither nausea nor spontaneous vomiting occurred. Given the lack of dosage information, the significance of such differences is questionable. As with many toxins, the expected survival time is inversely proportional to the dose. This has been specifically noted by Boyd and Taylor (1971) for rats and may have partially contributed to the increased survival time of the protein-deficient rats noted above. In four fatal human poisonings, death occurred between 6 and 11 hours after ingestion. This relatively short period prior to death may indicate that relatively massive doses were consumed. Specific lethal dose information, however, is available on only one child. In this case, the seventeen month old child is said to have consumed one-half a tablespoon of 60 percent toxaphene (McGee et al., 1952). Assuming a body weight of 14 kg, this would equal a dose of about 100 mg/kg.

Additional reports of oral poisoning in humans have not been encountered in the published literature. A draft of a report on poisoning incidents involving toxaphene compiled by the Pesticide Episode Response Branch, E.P.A. (1976) has recently become available. This report contains information voluntarily provided to the E.P.A. by various state, federal, and private organizations. Most of these individual reports, which vary widely in quality and detail, involve exposure to toxaphene along with a variety of other active agents. In no cases are estimates of toxaphene doses available. Of the 43 cases of human poisoning described in this report, four cases deal with accidental poisoning of children. Symptoms of poisoning are consistent with those described in the published reports: vomiting, convulsions, cyanosis, and coma. Two of the four cases were terminal. In a fifth case, an adult apparently attempted suicide by toxaphene ingestion. High levels of toxaphene were found in the blood, but the only symptom given is jaundice (Pesticide Episode Response Branch, 1976).

Pathology data on acute oral toxaphene poisoning are available only on rats (Table 6.10). The renal tubular damage is similar to that seen on subacute exposures. However, the fatty degeneration noted in Table 6.10 for acute intoxication does not correspond to the hydropic degeneration noted in Section 6.3.2.2 for subacute intoxication.

6.3.2.1.1.2 Acute dermal toxicity -- Based on the available information summarized in Table 6.11, toxaphene seems much less toxic on dermal exposures compared to oral exposures. However, the solvent used in dermal exposures may markedly affect toxicity estimates. Lehman (1952 b) noted only moderate skin irritation and hyperexcitability in rabbits after a single 24 hour exposure

Table 6.10. Summary of histopathologic findings at autopsy on albino rats which died from oral administration of toxaphene[a,b]

Organ	Histopathology
Adrenal glands	Cortical sinusoids frequently congested
Brain	Capillary-venous congestion and hemorrhage in the meninges; capillary congestion of the brain
Gastrointestinal tract	
Cardiac stomach	Granulocyte-infiltrated ulcers in late deaths
Pyloric stomach	Congestion of capillaries in the lamina propria near the mouths of the gastric glands
Small bowel	Capillary congestion of the villi
Cecum	Occasionally mild capillary congestion of the lamina propria
Colon	Normal appearance
Heart	Occasionally capillary congestion and capillary hemmorrhage
Kidneys	Cloudy swelling of the proximal and distal convoluted tubules and congestion of the loop of Henle
Liver	Extensive diffuse fatty degeneration and early necrosis
Lungs	Occasionally congestion and hemorrhage of the parenchyma
Muscle (ventral abdominal wall)	Normal appearance
Salivary (submaxillary) glands	Serous glands shrunken and ducts empty
Skin	Normal appearance
Spleen	Contracted red pulp
Testes	Varying degrees of inhibition of spermatogenesis
Thymus gland	Varying degrees of loss of thymocytes and capillary-venous congestion

[a] Source: Boyd and Taylor, 1971.

[b] Animals fed a low protein diet exhibited, in addition, signs of inhibited development of most organs and tissues.

Table 6.11. Acute dermal toxicity of toxaphene to laboratory mammals[a]

Organism	Vehicle	Dose (mg/kg)	Response	Dermal:Oral LD50 Ratio[b]	Reference
Rats Sherman, male, >90 days, >175 g, unfasted	Xylene	1075 (717-1613)	LD50 (95% Confidence Interval)	~12	Gaines, 1960 and 1969
Rats Sherman, female, >90 days, >175 g, unfasted	Xylene	780 (600-1014)	LD50 (95% Confidence Interval)	9.75	Gaines, 1960 and 1969
Rats	Xylene	930	LD50	~10	Hercules Inc., undated
Rabbits	Dust	>4000	LD50	---	Hercules Inc., undated
Rabbits	Peanut oil	<250	LD50	2.5-3.3	Hercules Inc., undated

[a] See Table 6.7 for dose-response data.
[b] Ratio of dermal toxicity to comparable oral toxicity from Table 6.7.

to toxaphene (dry, waxy form) at 4000 mg/kg. In contrast, toxaphene in 20 percent dimethyl phthalate caused tremors leading to convulsions and death at doses of less than 780 mg/kg. A similar solvent dependence is apparent in the rabbit studies summarized in Table 6.11. Gaines (1960, 1969) specifically designed his experimental protocol so that the oral and dermal toxicity of a variety of pesticides could be reliably compared (Table 6.7, Figure 6.2). In these studies, Gaines (1960, 1969) noted that compounds with oral and dermal toxicities on the same order of magnitude are most likely to present an occupational hazard to man. The relatively high ratio of dermal to oral toxicity for toxaphene suggests that this pesticide is not likely to present such a hazard.

This tentative assumption may be true for toxaphene, at least in cases of acute dermal exposure. Only one case of human poisoning by cutaneous absorption has been published and, based on the available details, the symptoms that developed cannot be unequivocally attributed to toxaphene. In this instance, a 70 year old male had his hands in contact with a toxaphene-lindane solution for two hours. After 10 hours, the following symptoms developed: headache, poor coordination, lassitude, severe nausea, and vomiting. Over the next week, this individual exhibited mild hyperthermia, flaccid musculature, and decreased response to stimuli. Only after 9 days did the individual become semicomatose. At no time were convulsions or hyperreflexia noted (Pollock, 1958). These signs and symptoms are not characteristic of toxaphene or lindane poisoning (Matsumura, 1975) and differ markedly from the previously described cases of acute oral toxaphene poisoning in humans. While clinical signs of intoxication may be expected to show some variation with different routes of entry, such profound variation is uncommon with the chlorinated insecticides. Gaines (1960, 1969) noted no difference between signs of intoxication in rats orally and dermally exposed to a variety of pesticides. Lackey (1949 a, b) similarly notes no remarkable differences in the response of dogs to subacute oral and dermal doses of toxaphene (Section 6.3.2.2). However, two cases of acute aplastic anemia associated with dermal exposure to toxaphene/lindane mixtures have been reported (Pesticide Episode Response Branch, 1976). One of these cases resulted in death due to acute myelomonocytic leukemia which was presumed to be secondary to the development of aplastic anemia. A recent review by the National Academy of Sciences (1977) links over 50 cases of aplastic anemia with exposure to lindane or benzene hexachloride, although no firm causal relationship could be demonstrated. Consequently, the role of toxaphene in the development of acute aplastic anemia is uncertain.

Hayes (1963) has estimated that a single dermal application of 46 g or daily applications of 2.4 g "over a period of days is very dangerous" to humans. For a 70 kg man, the single dose estimate is approximately 660 mg/kg. Documentation of this estimate is not provided by Hayes (1963).

6.3.2.1.1.3 Other routes of entry -- Very little information is available on the toxicity of technical grade toxaphene by routes other than oral and dermal exposure (Table 6.12). The acute intraperitoneal LD50 for mice approaches the lower level of the oral LD50 estimates for mammals (Table 6.8), suggesting that incomplete absorption may be a major factor in the variability of the oral LD50 estimates. Unlike oral exposures, intravenous injections of toxaphene to curarized animals cause a pronounced increase in blood pressure indicative of vasomotor stimulation (Conley, 1952). Thus, the lower LD50's with intravenous injections may be associated with a different mode of action caused by high toxaphene blood levels not attained with other routes of administration.

Table 6.12. Acute toxicity of toxaphene on inhalation, intravenous, and intraperitoneal injections

Organism	Route	Vehicle	Dose	Response	Reference
Mice	Inhalation	Oil-based mists	0.2 mg/100 cc air/ min x 2 hrs	LC50 estimate	Conley, 1952
Rats	Inhalation	40% dust	3.4 g/1 air x 1 hour	50% mortality	Hercules Inc., undated
Mice, male 18–20 g	Intraperitoneal injection	Dimethyl sulfoxide, 100 µl/mouse	42 mg/kg	LD50	Khalifa et al., 1974
Rats	Intravenous injection	Peanut oil and acacia, 1% toxaphene	13 mg/kg	LD50	Shelanski and Gellhorn, undated
Mammals	Intraperitoneal injection	N.S.	10–15 mg/kg	LD50 range	Conley, 1952

The inhalation LD50 value cited by Conley (1952) is based on exposures of mice to 0.2 mg/100 cc air/minute for varying periods of time. During exposure periods of 112, 116, and 158 minutes, mice mortality was 40, 100, and 60 percent, respectively. Because no significant lung damage was noted in fatally exposed mice, death was attributed to toxaphene absorption rather than to the oil-based mist used as the vehicle. No cases of acute inhalation poisoning in humans have been reported.

Toxaphene, as a 20 percent solution in kerosene, applied to eyes of rabbits and guinea pigs at doses of 2 mg/kg and 10 mg/kg, respectively, for 14 days caused only transient eye irritation with no permanent eye injury (Hercules Inc., undated).

6.3.2.1.2 Toxaphene fractions and components -- As discussed previously (Section 2.0), toxaphene may be separated into various fractions with each fraction containing a complex mixture of several toxaphene components. The chemical identity of these components has been determined in only a few instances. Toxicity data on toxaphene fractions and a few of the identified components are summarized in Tables 6.13 and 6.14. To emphasize that the fractions separated by Ohsawa and coworkers (1975) are chemically different from those of Seiber and coworkers (1975), fractions isolated by the former group are identified by Roman numerals (I to VII), and those of the latter by Arabic numeral (1 to 8). As previously defined (Section 2.0), the specific compounds isolated by Casida at al. (1974) and Turner et al. (1975) are referred to as toxicants A and B.

In the seven toxaphene fractions tested by Ohsawa and coworkers (1975), there is no apparent relationship of toxicity to either chlorine content or

Table 6.13. Acute intraperitoneal LD50's of toxaphene and toxaphene fractions to male mice[a]

	LD50 (mg/kg)[b]	Toxicity relative to toxaphene[c]	% Cl
Technical Toxaphene	42	1.0	68.6
Expected Values of Fractions I-VII[d]	45	0.93	60.8
Fraction I	252	0.16	68.1
Fraction II	110	0.38	68.3
Fraction III[e]	16	2.63	68.8
Fraction IV[e]	25	1.68	68.1
Fraction V[e,f]	52	0.81	66.8
Fraction VI[f]	63	0.67	64.1
Fraction VII	194	0.22	21.2
Toxicant A[g]	3.1	13.55	
Toxicant B[h]	6.6	6.36	

[a] Source: Modified from Khalifa et al., 1974 and Ohsawa et al., 1975.

[b] In 100 microliters of dimethyl sulfoxide per dose.

[c] LD50 of toxaphene ÷ LD50 of component.

[d] Expected value calculated as harmonic mean, i.e. $(X)_H = N/\Sigma(1/Xi)$.

[e] Includes Toxicant A.

[f] Includes Toxicant B.

[g] Toxicant A: Identified as mixture of 2,2,5-*endo*, 6-*exo*, 8,8,9,10-octachloroborane and 2,5,5-*endo*, 6-*exo*, 8,9,9,10-octachloroborane (Turner et al., 1975).

[h] Toxicant B: Identified as 2,2,5-*endo*, 6-*exo*, 8,9,10-heptachloroborane (Casida et al., 1974).

Table 6.14. Acute intraperitoneal LD50's of toxaphene and toxaphene fractions to mice[a]

	LD50 (mg/kg)[b]	Toxicity relative to toxaphene[c]	% (w/w) technical toxaphene
Technical Toxaphene	32	1.0	
Expected Values of Fractions 2-8[d]	41.4	0.77	
Fraction 1	---	---	2.1
Fraction 2	29	1.10	38.1
Fraction 3[e]	23	1.39	33.9
Fraction 4	65	0.49	12.0
Fraction 5	95	0.34	8.0
Fraction 6	79	0.41	3.1
Fraction 7	53	0.60	1.5
Fraction 8[f]	177	0.18	2.2

[a] Source: Seiber et al., 1975.
[b] In dimethyl sulfoxide (amount unspecified).
[c] LD50 of toxaphene ÷ LD50 of component.
[d] Expected value calculated as $\Sigma(\mathrm{LD50}_i \cdot \%\text{ of toxaphene } i)$.
[e] Contains Toxicant B.
[f] Major component of fraction identified as 2,5,6-exo-8,8,9,10-heptachlorodihydrocamphene.

the presence of toxicants A or B. The least toxic fraction, I, has about the same chlorine content as the most toxic fractions, III and IV. While both of these highly toxic fractions contain toxicant B, fraction V, which contains both toxicants A and B, is less toxic than technical grade toxaphene. The average LD50 computed as the harmonic mean of the seven fractions suggests an additive rather than synergistic relation among these fractions (Ohsawa et al., 1975). Similar patterns have been noted in the toxicity of these fractions and components to house flies (Section 5.6). As discussed in Section 6.2.3, differences in the toxicity of these components cannot be directly attributed to differences in detoxication.

The toxaphene fractions separated by Seiber and coworkers (1975) have a range of toxicity (Table 6.14) comparable to those noted in Table 6.13. While no definite pattern is apparent in the toxicity of the various fractions, high toxicity was associated with those fractions eluted by relatively apolar solvents - i.e., fractions 2 and 3 by 40 percent and 50 percent benzene in hexane. These fractions also comprised significantly greater percentages of the original technical toxaphene. As with the highly toxic fractions, III and IV, separated by Ohsawa and coworkers (1975), toxicant B was found by Seiber and coworkers (1975) in the fraction with the highest relative toxicity, fraction 3. Consistent with the results of Ohsawa et al. (1975), the expected value for the LD50 of fractions 2 to 8 does not indicate any synergistic effect among the toxaphene fractions.

Landrum and coworkers (1976) have recently determined the toxicity of two toxaphene column fractions to mice on intraperitoneal injection. Column fraction 3 had an LD50 of 38 (32 to 45) mg/kg which was not significantly different from that found for technical toxaphene, 33 (27 to 41) mg/kg. However, the other column fraction, designated column fraction 7, was only about one-half as toxic as toxaphene, having an LD50 of 67 (58 to 78) mg/kg. About one-half of this latter fraction consisted of 2,5,6-exo,8,8,9,10-heptachlorodihydrocamphene, which has been shown to be significantly less toxic than technical toxaphene to houseflies (Section 5.6.2.2.1.2).

6.3.2.2 Subacute Toxicity -- Table 6.15 summarizes the effects of subacute oral administration of toxaphene to laboratory mammals. Except for convulsions observed in dogs given 5 mg/kg/day, none of the exposures detailed in Table 6.15 resulted in clinical signs of toxaphene poisoning. The ability of dogs to tolerate large cumulative doses (176 to 424 mg/kg) when given at 4 mg/kg/day suggests a rather sharp threshold level for central nervous system stimulation. This is consistent with information discussed in Section 6.2.4, showing that toxaphene is eliminated relatively rapidly. A similar pattern is seen in rats on intraperitoneal injection. Ohsawa and coworkers (1975) have found that male rats injected with 50 mg toxaphene (approximately 300 mg/kg) every 48 hours tolerated cumulative doses of 700 to 2000 mg/kg (over ten times the single oral LD50 dose) before marked lethality occurred.

In subacute exposures which do not cause apparent central nervous system stimulation, no increases in mortality are noted. However, pathological changes of the kidneys and liver, as well as changes in blood chemistry, seem to be common features of subclinical toxaphene intoxication.

Ortega and coworkers (1957) (using rats) and Lackey (1949 a) (using dogs) have noted similar changes in liver histology. Morphologically, these changes appear as vacuoles of plasma with occasional red blood cells found within

Table 6.15. Subacute oral toxicity of toxaphene

Organism	Vehicle	Duration	Dose (mg/kg/day or ppm in diet)	Estimated cumulative dose (mg/kg)	Response[a]	Reference
Mice, both albino and wild strains	Diet	Several weeks or months	50 mg/kg/day (250–480 ppm)	>300	Changes in blood chemistry and urine protein	Baeumler, 1975
Rats	Diet	12 weeks	189 ppm		No apparent adverse effects	Clapp et al., 1971
Rats	N.S.[b]	7 months	1.2–4.8 mg/kg/day	~250–1000	Temporary change in blood chemistry	Grebenyuk, 1970
Rats, Sherman, male and female, ~100 g	Diet	2–9 months	50 and 200 ppm		Questionable liver pathology	Ortega et al., 1951
Rats and guinea pigs	Diet	6 months	100–800 ppm		No significant effect	Shelanski and Gellhorn, undated
Dogs	Corn oil	"A few days"	5 mg/kg/day	~15–35	Convulsion	Lackey, 1949 a
	Corn oil	44 days	4 mg/kg/day	176	Questionable liver pathology; renal tubular degeneration	Lackey, 1949 a
	Corn oil	106 days	4 mg/kg/day	424	Questionable liver pathology; renal tubular degeneration	Lackey, 1949 a

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[a] See text for details.
[b] N.S. - not specified.

hepatic cells. This condition, referred to as hydropic accumulation, is distinct from fatty degeneration. In neither rats nor dogs was hydropic accumulation associated with the destruction of hepatic cells. However, Ortega and coworkers (1957) also noted occasional masses of red blood cells invading the cytoplasm of liver cells in areas of hypertrophy and margination. In addition to liver damage, Lackey (1949 a) also noted wide-spread degeneration of the tubular epithelium, occasionally accompanied by inflammation of the pelvis of the kidney. Identical pathological changes were seen in dogs surviving prolonged dermal exposures to toxaphene (Lackey, 1949 b).

As noted in Table 6.15, alterations in clinical chemistry have also been seen in subacute oral toxaphene exposures. Mice with no clinical signs of intoxication evidenced consistent increases in serum acid phosphatase, glutamic-pyruvic transaminase, and gamma-glutyamyl transpeptidase activities, along with increased neutrophil counts and changes in urine protein (Baeumler, 1975). At a much lower daily dose, rats had only a transient increase in serum alkaline phosphatase during the fifth month of intoxication and showed no variation in urine hippuric acid (Grebenyuk, 1970). Increases in all of the above enzyme activities are consistent with the mild liver pathology associated with subacute toxaphene exposure.

Lehman (1952 b) states that the 90 day dermal LD50 of toxaphene (as a dry wax) is 40 mg/kg in rabbits. No details of symptoms or pathology are provided.

The subacute inhalation toxicity of toxaphene is summarized in Table 6.16. Details of these studies were not available for this review. Hercules Inc. (undated) indicates that, unlike subacute oral exposures, these inhalation exposures did not result in abnormal blood chemistry or hematologic measurements.

Table 6.16. Subacute inhalation toxicity of toxaphene[a,b]

Organism	Concentration (mg/liter air)	Form	Duration	Response
Rat	0.25	Dust	1 week	100% mortality
Rats and rabbits	0.04	Mist	3 weeks	No observed effects
Rats and rabbits	0.20	Mist	3 weeks	No observed effects
Rats	0.50	Mist	3 weeks	No observed effects
Rats, dogs, and guinea pigs	0.004	Dust	3 months	No observed effects
Rats	0.012	Dust	3 months	No mortality
Dogs	0.012	Dust	3 months	50% mortality
Guinea pigs	0.012	Dust	3 months	No mortality
Rats	0.04	Dust	3 months	27% mortality
Dogs	0.04	Dust	3 months	67% mortality
Guinea pigs	0.04	Dust	3 months	20% mortality

[a] Source: Hercules Inc., undated.
[b] All exposures for 6 hours/day, 5 days/week.

The most pronounced effect prior to death was severe weight loss. As in subacute oral exposures, female rats had slight focal cellular necrosis of the liver.

Hercules Inc. (undated) has conducted controlled dermal and inhalation exposures of humans to toxaphene. Toxaphene doses of 300 mg/day applied to the skin of 50 volunteers for 30 days produced no gross toxic effects. Similarly, cotton patches treated with toxaphene produced neither sensitization nor primary skin irritation when applied to the skin of 200 subjects. In daily 30 minute inhalation exposures to toxaphene mists of 500 mg/cu m air, humans exposed for ten consecutive days followed by three daily exposures three weeks later showed no adverse effects based on physical examinations as well as blood and urine tests (Shelanski, 1974).

However, Warraki (1963) has attributed two cases of acute bronchitis with military lung shadows to inhalation of toxaphene during applications of toxaphene spray. Both individuals, male adults, had been exposed to toxaphene sprays from 1 1/2 to 2 months before the onset of pulmonary insufficiency. Maximum breathing capacity was between 19 and 22 percent of normal. All adverse effects, including pulmonary lesions, were reversible within three months after toxaphene exposure was discontinued. No central nervous system effects were noted. One case of allergic rhinitis in a worker exposed to toxaphene by inhalation has been reported. However, details on the duration of exposures were not given (Pesticide Episode Response Branch, 1976).

6.3.2.3 Chronic Toxicity -- Long term exposures to low dietary levels of toxaphene are summarized in Table 6.17. All studies note some form of liver pathology in rats at dietary levels of 100 ppm or above. At 100 ppm, cytoplasmic

Table 6.17. Chronic toxicity of toxaphene at low dietary levels to laboratory mammals

Organism	Duration of feeding	Toxaphene concentration in diet	Response[a]	Reference
Rats, Sprague Dawley	3 generations	25 ppm	No effect	Kennedy et al., 1973
		100 ppm	Liver pathology	
Rats	Lifetime	25 ppm	No effect	Lehman, 1952 a
		100 ppm	Liver pathology	
Rats	Lifetime	25 ppm	Liver pathology	Fitzhugh and Nelson, 1951
Rats	2 years	25 ppm	No effect	Hercules Inc., undated
	2 years	100 ppm	Slight liver damage	
		1000–1600 ppm	CNS stimulation	
Dogs	2 years	5–20 ppm	No effect	Hercules Inc., undated
Dogs	2 years	40 ppm	Slight liver degeneration	
		200 ppm	Moderate liver degeneration	
Dogs	1360 days (~3.7 years)	5 mg/kg/day[a]	Liver necrosis	Hercules Inc., undated
Monkeys	2 years	10–15 ppm (~0.64-0.78 mg/kg/day)	No clinical or histological effects	Hercules Inc., undated

[a] Administered in capsules containing toxaphene dose in corn oil; 5 mg/kg/day equivalent to 200 ppm in diet.

vacuolization similar to that seen on subacute oral exposure was noted by Kennedy and coworkers (1973). Lehman (1952 a) noted both cytoplasmic vacuolization and fatty degeneration of the liver in rats fed 100 ppm. At 25 ppm, Fitzhugh and Nelson (1951) did observe increased liver weight with minimal liver cell enlargement. Unpublished studies on rats, dogs, and monkeys by Hercules Inc. (undated) are in general agreement with the above published reports. The lowest dietary level of toxaphene producing unequivocal liver damage over a two year feeding period is 20 ppm. Only at relatively high concentrations - i.e., 1000 ppm - does chronic toxaphene exposure elicit central nervous system effects characteristic of acute intoxication. The relevance of these studies to other aspects of toxaphene toxicology is discussed in subsequent sections.

No cases of chronic human intoxication have been encountered in the literature. In an epidemiological survey of workers exposed to toxaphene and a variety of other pesticides, no evidence of abnormal heme synthesis or abnormal sympathoadrenal activity - based on urinary analysis of unconjugated catecholamines - has been noted (Embry et al., 1972).

6.3.2.4 Carcinogenicity -- None of the chronic feeding studies detailed in Section 6.3.2.3 noted any increase of tumor incidence in the experimental animals. However, a closely related pesticide, Strobane (chlorinated terpenes), has been shown to cause an increase in tumor production in male but not female mice (Table 6.18, Innes et al., 1969). In this study, two strains of mice were used and designated strain X (C57BL/6xC3H/Anf,F1) and strain Y (C57BL/6xAKR,F1). Seven days after birth, the mice received 4.64 mg/kg/day of Strobane in 0.5 percent gelatin by intubation until the mice were four

Table 6.18. Tumors in mice associated with oral administration of strobane[a]

	Strain	Number of mice necroscopied (M)	(F)	Hepatomas (M)	(F)	Pulmonary tumors (M)	(F)	Lymphomas (M)	(F)	Total tumors (M)	(F)	Sum
Number of tumors	X	15	18	2	0	1	1	5	2	8	3	
	Y	18	18	11	0	0	0	0	0	11	0	
Relative Risk compared to positive control	X	--	--	1.56	0	1.40	2.07	7.10[b]	2.81	2.90	2.11	2.56[c]
	Y	--	--	23.84[b]	0	0	0	0	0	6.92[b]	0	4.73[b]
Relative Risk compared to positive control	X	--	--	0.136	0					0.550	0.099	.265
	Y	--	--	2.04	0					1.110	0	.704

[a] Source: Modified from Innes et al., 1969.
[b] Increased tumor yield significant at 0.01 level.
[c] Increased tumor yield significant at 0.05 level.

weeks old. The dose was calculated by the weight of the seven day old mice and not adjusted for weight gain. At age four weeks, Strobane was administered in the food at a concentration of 11 ppm. Based on the weight and food consumption at four weeks, this concentration resulted in daily doses of about 4.6 mg/kg. This schedule was maintained for eighty weeks, using thirty-six mice of each strain evenly divided by sex. Male mice of strain X developed a significantly (p less than 0.01) higher incidence of lymphomas. Males of strain Y, however, showed significant (p less than 0.01) increases only in hepatomas.

In this study, hepatomas are classified as liver tumors which have not metastasized. Such a classification does not imply "that these tumors are benign. Indeed, it seems more reasonable to conclude that the great majority had malignant potential" (Innes et al., 1969). The positive control consisted of combined data from mice exposed to seven known carcinogens: urethane, ethylene imide, amitrol, aramite, dihydrosafrole, isosafrole, and safrole. Although the increased incidence of hepatomas in male mice of strain Y administered Strobane compared to the positive control has no absolute definitive value, it is indicative of highly positive tumorigenic potential.

Under contract to the National Cancer Institute, Gulf Research Institute has recently completed a carcinogenicity bioassay of toxaphene (National Cancer Institute, 1979). It should be noted that this study, which was conducted from 1971 to 1973, did not follow current NCI protocols (NCI, 1977). Specifically, only ten organisms were used in matched control groups, and matched-fed control groups were not utilized. In this study, groups of Osborne-Mendel rats and B6C3F1 hybrid mice were exposed to technical-grade toxaphene in the diet for 80 weeks. Details of the dose schedule and number of organisms used are provided in Tables 6.19 and 6.20.

Table 6.19. Toxaphene chronic feeding studies in rats[a]

Sex and Test Group	Initial No. of Animals[b]	Toxaphene in diet[c] (mg/kg)	Time on Study: Dosed[d] (weeks)	Time on Study: Observed[e] (weeks)	Time-weighted Average dose[f] (mg/kg)
MALE					
Matched-control	10	0		108-109	
Low-dose	50	1,280	2		556
		640	53		
		320	25		
		0		28	
High-dose	50	2,560	2		1,112
		1,280	53		
		640	25		
		0		28	
FEMALE					
Matched-control	10	0		108-109	
Low-dose	50	640	55		540
		320	25		
		0		30	
High-dose	50	1,280	55		1,080
		640	25		
		0		30	

[a] National Cancer Institute, 1979.

[b] All animals were 5 weeks of age when placed on study.

[c] Initial doses shown were toxic; therefore, doses were lowered after 2 weeks and again at 53 or 55 weeks, as shown.

[d] All animals were started on study on the same day.

[e] When diets containing toxaphene were discontinued, dosed rats and their matched controls were fed control diets without corn oil for 20 weeks, then control diets (2% corn oil added) for an additional 8 weeks.

[f] Time-weighted average dose $= \frac{\Sigma(\text{dose in ppm x no. of weeks at that dose})}{\Sigma(\text{No. of weeks receiving each dose})}$

Table 6.20. Toxaphene chronic feeding studies in mice[a]

Sex and Test Group	Initial No. of Animals[b]	Toxaphene in diet[c] (mg/kg)	Time on Study: Dosed[d] (weeks)	Time on Study: Observed[e] (weeks)	Time-weighted Average dose[f] (mg/kg)
MALE					
Matched-control	10	0		90-91	
Low-dose	50	160	19		99
		80	61		
		0		11	
High-dose	50	320	19		198
		160	61		
		0		10	
FEMALE					
Matched-control	10	0		90-91	
Low-dose	50	160	19		99
		80	61		
		0		11	
High-dose	50	320	19		198
		160	61		
		0		10	

[a] National Cancer Institute, 1979.

[b] All animals were 5 weeks of age when placed on study.

[c] Initial doses shown were toxic; therefore, doses were lowered at 19 weeks, as shown.

[d] All animals were started on study on the same day.

[e] When diets containing toxaphene were discontinued, dosed mice and their matched controls were fed control diets without corn oil for 7 weeks, then control diets (2% corn oil added) for an additional 3 to 4 weeks.

[f] Time-weighted average dose $= \frac{\Sigma(\text{dose in ppm x no. of weeks at that dose})}{\Sigma(\text{No. of weeks receiving each dose})}$

Toxaphene was added to the feed in acetone. In addition, 2 percent corn oil was added to the diet as a dust suppressant. Actual dietary toxaphene concentrations, which were confirmed by gas-liquid chromatography, did not deviate from the nominal concentration by more than 6.9 percent. In addition to the matched control groups indicated in these tables, pooled control groups were used in the statistical analyses. For rats, pooled controls consisted of matched controls from similar bioassays on captan, chloraben, lindane, malathion, and picloram, as well as the matched controls from the toxaphene bioassay. For mice, pooled controls consisted of matched controls from similar bioassays on lindane, malathion, phosphamidon, and tetrachlorvinphos, as well as the matched controls from the toxaphene study. Organisms used in all pooled control groups were of the same strains, from the same suppliers, and examined by the same pathologists.

During the course of this study, both rats and mice evidenced signs of general toxic effects. Both male and female rats in the high-dose group developed body tremors at week 53. From week 52 to week 80, other clinical signs, which occurred primarily in toxaphene-dosed rats, included diarrhea, dyspnea, pale mucous membranes, alopecia, rough hair coats, dermatitis, ataxia, leg paralysis, epistaxis, hematuria, abdominal distention, and vaginal bleeding. Female rats in both dose groups had lower mean body weights than the matched controls. No dose-related effect on mortality was noted in any of the rat test groups. In mice, males and females in each dose group displayed a significant increase in mortality when compared to the matched controls. In high-dose male mice, mean body weights were generally lower than those in the matched control group. Clinical signs of toxicity in mice included abdominal distention, diarrhea, alopecia, rough hair coats, and dyspnea.

The effects of dietary toxaphene on tumor incidence in male rats, female rats, male mice, and female mice are summarized in Tables 6.21, 6.22, 6.23, and 6.24, respectively.

In male rats in the high dose group, a significant increase was noted in the incidence of follicular-cell carcinomas or adenomas of the thyroid. Of the nine thyroid tumors which were found in this group, two were carcinomas. A significant increase of follicular-cell adenomas of the thyroid was also noted in the high-dose group of female rats. No carcinomas of the thyroid were found in this group. In both of these groups, the development of thyroid tumors was dose-related. A significant increase was also noted in the incidence of chromophobes, adenomas, chromophobe carcinomas, and adenomas of the pituitary in the high-dose group female rats. However, an examination of historical control data on the incidence of pituitary tumors in female rats suggested that an association between the administration of toxaphene and the development of pituitary tumors could not be maintained based on the results of this study.

In both male and female mice, significant increases were noted in the incidence of hepatocellular carcinomas and in the incidence of hepatocellular carcinomas combined with neoplastic nodules of the liver.

Based on the results of this study, the National Cancer Institute has concluded that "Toxaphene was carcinogenic in male and female B6C3F1 mice, causing increased incidences of hepatocellular carcinomas. The test results also suggest carcinogenicity of toxaphene for the thyroid of male and female Osborne-Mendel rats" (National Cancer Institute, 1979).

6.3.2.5 Mutagenicity -- Epstein et al. (1972) have used a modified dominant lethal assay in mice to evaluate the mutagenic potential of a variety of chemical agents including toxaphene. In this study, four groups of male ICR/Ha Swiss mice

Table 6.21. Analyses of the incidence of primary tumors in male rats fed toxaphene in the diet[a,b]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Neoplastic Nodule[c]	1/9 (11)	1/52 (2)	6/44 (14)	4/45 (9)
P Values[d]	N.S.	N.S.	P = 0.034**	N.S.
Weeks to First Observed Tumor	109	--	108	94
Pituitary: Chromophobe Adenoma, Carcinoma, NOS, or Adenoma, NOS[c]	3/7 (43)	8/46 (17)	13/42 (31)	5/31 (16)
P Values[d]	N.S.	N.S.	N.S.	N.S.
Weeks to First Observed Tumor	102	--	85	95
Adrenal: Adenoma, NOS, Cortical Adenoma, or Carcinoma	4/9 (44)	5/52 (10)	5/41 (12)	3/37 (8)
P Values[d,e]	P = 0.019 (N)	N.S.	P - 0.043 (N)*	P - 0.020 (N)*
Weeks to First Observed Tumor			85	85
Spleen: Hemangioma[c]	0/9 (0)	0/49 (0)	3/45 (7)	3/42 (7)
P Values[d]	N.S.	N.S.	N.S.	N.S.
Weeks to First Observed Tumor	--	--	83	85
Thyroid: Follicular-cell[f] Carcinoma or Adenoma[c]	1/7 (14)	2/44 (5)	7/41 (17)	9/35 (26)
P Values[d]	N.S.	P - 0.007	N.S.	P - 0.008**
Weeks to First Observed Tumor	109	--	104	56

[a] National Cancer Institute, 1979.

[b] Dosed groups received time-weighted average doses of 556 or 1,112 ppm.

[c] Number of tumor-bearing animals/number of animals examined at site (percent).

[d] Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparisons of that dosed group with the matched-control group (*) or with the pooled-control group (**) when P less than 0.05 for either control group; otherwise, not significant (N.S.) is indicated.

[e] A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

[f] Data being reevaluated. See Test.

Table 6.22. Analyses of the incidence of primary tumors in female rats fed toxaphene in the diet[a,b]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Integumentary System: Malignant Fibrous Histiocytoma of the Subcutaneous Tissue[c]	0/10 (0)	0/55 (0)	1/50 (2)	3/49 (6)
P Values[d]	N.S.	N.S.	N.S.	N.S.
Weeks to First Observed Tumor	--		105	83
Mammary Gland: Fibroadenoma[c]	0/10 (10)	6/55 (11)	10/50 (20)	10/49 (20)
P Values[d]	N.S.	N.S.	N.S.	N.S.
Weeks to First Observed Tumor	87	--	19	67
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[c]	1/10 (10)	1/55 (2)	5/42 (12)	4/40 (10)
P Values[d]	N.S.	N.S.	N.S.	N.S.
Weeks to First Observed Tumor	109	--	108	109
Pituitary: Chromophobe Adenoma, Carcinoma, or Adenoma, NOS[c]	3/8 (38)	17/51 (33)	15/41 (37)	23/39 (59)
P Values[d]	P = 0.046	P = 0.012	N.S.	P = 0.013**
Weeks to First Observed Tumor	85	--	75	79
Thyroid: Follicular-celle Adenoma[c]	0/6 (0)	1/46 (2)	1/43 (2)	7/42 (17)
P Values[d]	P = 0.022	P = 0.008	N.S.	P = 0.021**
Weeks to First Observed Tumor	--	--	102	105

Table 6.22. Analyses of the incidence of primary tumors in female rats fed toxaphene in the diet[a,b] (Cont'd)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Adrenal: Cortical Adenoma or Carcinoma[c]	0/8 (0)	3/50 (6)	3/44 (7)	6/43 (14)
P Values[d]	N.S.	N.S.	N.S.	N.S.
Weeks to First Observed Tumor	--	--	104	87
Uterus: Endometrial Stromal Polyp[b]	0/9 (0)	5/53 (9)	9/41 (22)	5/45 (11)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Weeks to First Observed Tumor	--	--	87	109

[a] National Cancer Institute, 1979.

[b] Dosed groups received time-weighted average doses of 540 or 1,080 mg/kg.

[c] Number of tumor-bearing animals/number of animals examined at site (percent).

[d] Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group (*) or with the pooled-control group (**) when P less than 0.05 for either control group; otherwise, not significant (N.S.) is indicated.

[e] Data being reevaluated. See text.

Table 6.23. Analyses of the incidence of primary tumors in male mice fed toxaphene in the diet[a,b]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[c]	0/10 (0)	4/48 (8)	34/49 (69)	45/46 (98)
P Values[d]	P less than 0.001	P less than 0.001	P less than 0.001* P less than 0.001**	P less than 0.001* P less than 0.001**
Weeks to First Observed Tumor	--	--	73	59
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[c]	2/10 (20)	7/48 (15)	40/49 (82)	45/46 (98)
P Values[d]	P less than 0.001	P less than 0.001	P less than 0.001* P less than 0.001**	P less than 0.001* P less than 0.001**
Weeks to First Observed Tumor	90	--	73	59

[a] National Cancer Institute, 1979.

[b] Dosed groups received time-weighted average doses of 99 or 198 mg/kg.

[c] Number of tumor-bearing animals/number of animals examined at site (percent).

[d] Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group (*) or with the pooled-control group (**) when P less than 0.05 for either control group; otherwise, not significant (N.S.) is indicated.

Table 6.24. Analyses of the incidence of primary tumors in female mice fed toxaphene in the diet[a,b]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[c]	0/9 (0)	0/48 (0)	5/49 (10)	34/49 (69)
P Values[d]	P less than 0.001	P less than 0.001	P = 0.030**	P less than 0.001
				P less than 0.001**
Weeks to First Observed Tumor	--	--	89	72
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[c]	0/9 (0)	0/48 (0)	18/49 (37)	40/49 (82)
P Values[d]	P less than 0.001	P less than 0.001	P = 0.026*	P less than 0.001*
			P less than 0.001**	P less than 0.001**
Weeks to First Observed Tumor	--	--	89	72

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[a] National Cancer Institute, 1979.

[b] Dosed groups received time-weighted average doses of 99 or 198 mg/kg.

[c] Number of tumor-bearing animals/number of animals examined at site (percent).

[d] Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group (*) or with the pooled-control group (**) when P less than 0.05 for either control group; otherwise, not significant (N.S.) is indicated.

were given toxaphene intraperitoneally - single doses of 36 mg/kg and 180 mg/kg - and orally - five doses of 8 mg/kg/dose and 16 mg/kg/dose. After dosing, the treated males were mated to groups of untreated females over an 8-week period. Based on measurements of early fetal deaths per pregnancy and the percent of females with early fetal deaths, the toxaphene-treated groups did not differ significantly from controls. Thus, in this strain of mice, toxaphene apparently does not produce chromosomal abnormalities that preclude zygote development.

Hill (1977) has summarized information on the mutagenicity testing of toxaphene in bacterial systems. Ames tests have been conducted on Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 with and without metabolic activation by non-induced mammalian liver fractions. Positive results were obtained for strains TA-98 (frameshift mutation) and TA-100 (base pair substitution) only in tests without metabolic activation. All other tests were negative. A "high temperature" toxaphene has elicited positive dose response increases in strains TA-98 and TA-100 only with metabolic activation. All the above tests were conducted by Litton Bionetics Inc. for Hercules Inc.

In addition to these studies, work has been conducted on the mutagenicity of toxaphene in the Salmonella system by Dr. Kim Hooper of Bruce Ames' group in Berkeley, California (Hill, 1977). His results indicate that toxaphene and toxaphene subfractions are mutagenic to strain TA-100 with and without activation by Aroclor-induced rat microsomes. Mutagenic activity was decreased in those tests using microsomal activation.

A recently completed study by U.S. EPA (1978) found no significant differences in the rates of chromosomal aberrations in leukocytes between groups of individuals occupationally exposed to toxaphene and groups with no occupational exposures to toxaphene.

6.3.2.6 Teratogenicity -- In a study by Kennedy et al. (1973), male and female rats were fed dietary levels of 25 mg/kg diet and 100 mg/kg toxaphene. Gross and microscopic pathology of F3 weanlings revealed no indication of teratogenic effects. Further, no statistically significant variations from controls were noted in either dose group for any of the following parameters: mating index, fertility index, pregnancy index, parturition index, mean viable liter size, live birth index, 5-day survival index, lactation index, or weaning body weights of offspring. One of 16 females from each dose group resorbed an entire litter. This was not seen in any of the 32 control females but did occur in tests with another pesticide, delnav.

In multigeneration studies of mice given toxaphene at 25 mg/kg diet, no effects on fertility, gestation, viability, lactation, or survival indices were observed (Keplinger et al., 1970).

In addition to these long-term dietary studies, one study (Chernoff and Carver, 1976) has been conducted in which toxaphene in corn oil was administered to pregnant female rats and mice from days 7 to 16 of gestation at doses of 15, 25, and 35 mg/kg/day. All doses produced signs of maternal and fetal toxicity but no teratogenic effects.

DiPasquale (1977) has examined the effects of toxaphene on fetal guinea pig development. In this study, toxaphene was administered to pregnant females at a dose of 15 mg/kg body weight orally from day 21 to day 35 of gestation. No effects were noted on anatomical development of the fetus. The only sign of fetotoxicity was a decrease in collagen-containing structures. This was attributed to a functional deficiency of vitamin C related to mixed-function oxidase induction. Maternal guinea pigs showed a slight loss of body weight but no effects were seen on maternal liver weight or mortality.

6.3.2.7 Drug Interactions -- As indicated in Table 6.25, toxaphene offers a slight protective effect to rats when administered by intubation at equitoxic doses with three of seven tested pesticides (Keplinger and Deichmann, 1967). Because both pesticides were given at about the same time, the protective effect is probably not attributable to liver microsomal mixed-function oxidase induction. However, hepatic microsomal mixed-function oxidase induction may account for the protective effects of aldrin and dieldrin on toxaphene toxicity to rats noted by Deichmann and Keplinger (1970). In this study, the 96 hour LD50 of toxaphene to rats receiving no pretreatment was 78 mg/kg. In rats given single oral doses of aldrin or dieldrin at 30 mg/kg four days prior to toxaphene dosing, the 96 hour LD50's of toxaphene were 180 mg/kg and 165 mg/kg, respectively. This decrease in toxaphene toxicity corresponded to increased O-dealkylase and O-demethylase activities in the liver homogenates of pretreated animals. A decrease in toxaphene toxicity was also seen in rats after feeding on diets containing 400 ppm DDT for two weeks. In these animals, the 96 hour LD50 of toxaphene was 215 mg/kg, about three times that seen in untreated controls.

Toxaphene has been found to have no ability to supress pentylene tetarzole-induced convulsions in rats (Ghazal, 1965).

As discussed in Section 6.3.2.1.1.2, dermal exposure to toxaphene/lindane mixtures has been associated with the development of acute aplastic anemia in man. This observation, however, is based solely on rather limited epidemiological data.

Table 6.25. Expected and observed oral LD50's of toxaphene plus other pesticides in rats[a]

Other pesticide	Expected LD50 (mg/kg)	Observed LD50 (mg/kg)	Ratio E/O
Malathion	1000	820	1.22
Carbaryl	445	524	0.85
V-C 13	120	146	0.82
Delnav	90	112	0.80
Parathion	67	100	0.67[b]
Diazinon	150	227	0.66[b]
Trithion	65	121	0.54[b]

[a] Source: Keplinger and Deichmann, 1967.
[b] Ratio considered indicative of protective effects by toxaphene.

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7.0 ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

7.1 SUMMARY

Soil and water monitoring studies suggest that toxaphene is not as wide spread an environmental contaminant as other chlorinated hydrocarbon insecticides, such as DDT and dieldrin. Toxaphene is rarely detected in soil, water, or sediment samples that have not received direct applications of the pesticide for crop protection or rough fish control. This may be due, in part, to the difficulty encountered in trace analysis of such a complex mixture of compounds and the high volatility of toxaphene. Sampling of western North Atlantic Ocean air indicates that mean levels of toxaphene are equal to or twice those of PCB's and ten times higher than those of other pesticides such as DDT. This probably results from both the large scale use of toxaphene and its volatility. These results also suggest that toxaphene, which is detected near the island of Bermuda, has travelled 1200 km.

Degradation of toxaphene in the environment is poorly understood. Several researchers have noted that longer retention time (less volatile) peaks in the gas chromatogram of toxaphene residue decrease while shorter retention time (more volatile) peaks increase. Residues from water and sediment do not appear to be products of dehydrohalogenation. The rate of degradation varies considerably. One study determined that the concentration of toxaphene in sediment decreased by a factor of 2 every 20 days. However, concentrations in lakes treated 3 to 9 years before monitoring contained 1 to 4 micrograms per liter in water and 0.2 to 1 ppm in sediment. Adsorption to sediment and suspended solids appears to be an important process for removing toxaphene from water. The calculated half-life for evaporation of toxaphene from water is 10.4 hours.

The reported half-residence time for toxaphene in soil varies from 100 days to 11 years. The latter is considered to be a maximum value. Most studies suggest that little leaching of toxaphene through soil occurs, although one investigation found toxaphene in ground water below a treated plot.

7.2 MONITORING

7.2.1 Residues in Air

The highest toxaphene residues in air have been found in areas where it is applied for agricultural purposes (especially cotton production) (Arthur et al., 1976; Stanley et al., 1971; Tabor, 1965, 1966). Studies in cotton growing areas demonstrate that airborne residues are highest during the cotton growing season and decrease to low levels after harvesting, but spring tilling releases soil residues to the air. The recent identification of ng/cu m levels over the Atlantic Ocean, where toxaphene has not been applied, establishes that toxaphene residues move with air currents analagous to DDT (Bidleman et al., 1976; Bidleman and Olney, 1975).

Arthur et al. (1976) reported a three year (January 1972 to December 1974) study of toxaphene residues at Stoneville, Mississippi, which is located in the southern cotton belt. They sampled on a weekly schedule in which they collected at intervals of 4.29 minutes per hour (12 hours per week). They used a MISCO Model 88 air sampler containing ethylene glycol as the trapping solvent. Table 7.1 summarizes the average monthly toxaphene concentration for the three years. The highest average monthly concentrations were detected during July and August. The highest average weekly concentration was 1746.5 ng/cu m. Although they do not explain the sharp decrease in average residues for 1973 compared to 1972, they attribute the increase in 1974 over 1973, in part, to a 22 percent increase in toxaphene use.

Table 7.1. Average monthly atmospheric levels of toxaphene (ng/cu m) in Stoneville, Mississippi[a]

	1972	1973	1974
January	0.0	0.0	10.9
February	13.0	0.0	9.7
March	68.0	16.8	19.1
April	67.4	10.8	27.7
May	32.4	46.8	44.3
June	44.2	109.9	38.6
July	400.7	41.1	175.0
August	1540.0	268.8	903.6
September	827.9	322.6	524.6
October	97.9	161.1	114.8
November	9.3	0.0	32.[illegible]
December	0.0	9.9	12.6
Average	258.4	82.3	159.5

[a] Source: Arthur et al., 1976.

Stanley et al. (1971) examined toxaphene and 18 other pesticides and pesticide metabolites for six months at nine locations: Baltimore, Maryland (urban); Buffalo, New York (rural); Dothan, Alabama (rural); Fresno, California (urban); Iowa City, Iowa (rural); Orlando, Florida (rural); Riverside, California (urban); Salt Lake City, Utah (urban); and Stoneville, Mississippi (rural). The sites were sampled during 1967 and 1968; sites were examined at different times during this period. They sampled twice a month for seven day intervals, with air being collected for either 12 or 24 hours per day. Toxaphene was only detected at three rural locations in southern states. Table 7.2 lists the maximum levels and the number of samples containing toxaphene. Toxaphene residues on the first day of each sampling week at Stoneville, Mississippi, are summarized in Table 7.3.

Tabor (1965, 1966) monitored air in seven communities located in southern agricultural areas and three eastern urban areas. Toxaphene was detected only in communities located in the two cotton growing areas (Leland, Washington County, Mississippi, and Newellton, Texas). Toxaphene residues were not detected in the urban communities or the five rural communities located in fruit or vegetable growing areas. At Leland, toxaphene was detected in 6 of 15 samples collected from July to September 1963; concentrations ranged from 1.2 to 7.5 ng/cu m. Toxaphene was found in 6 of 10 samples taken during the same period at Newellton, Texas (3.1 to 15 ng/cu m).

Bidleman and Olney (1975) measured atmospheric toxaphene residues over the east coast of the U.S., near Bermuda, and over the open ocean (Western North Atlantic Ocean) during 1973 to 1974. They collected residues by drawing 200 to 3000 cu m of air through a device consisting of a Gelman A glass

Table 7.2. Maximum toxaphene levels found in air samples of nine localities[a]

Site	Number of total samples	Toxaphene range (ng/cu m)	Samples containing toxaphene
Baltimore	123	---	0
Buffalo	57	---	0
Dothan	90	27.3 - 79.0	11
Fresno	120	---	0
Iowa City	94	---	0
Orlando	99	20.0 - 2520	9
Riverside	94	---	0
Salt Lake City	100	---	0
Stoneville	98	16.0 - 111.0	57

[a] Source: Adapted from Stanley, 1968, and Stanley et al., 1971.

Table 7.3. Toxaphene found in air samples from Stoneville, Mississippi the first day of each sampling week[a]

Date	Toxaphene level, ng/cu m
August 14-16	283
August 21-22	373
September 11-12	701
October 2-3	161
July 1-2	68
July 15-16	116
July 29-30	62
August 12-13	135

[a] Source: Stanley et al., 1971.

fiber filter and a polyurethane foam plug (Section 2.6.2). Toxaphene was analyzed by standard gc procedures, both with and without alkaline dehydrochlorination (Section 2.5.2). Although toxaphene had weathered, the collected residues were clearly recognized. Figure 7.1 and Table 7.4 describe the sampling sites and measured residues. At Bermuda, 36 samples were collected: 26 when winds blew from inside a 90 E to 240 SW selected sector (ISS) (air passed over hundreds of kilometers of open ocean and not over the island of Bermuda); 7 when winds blew from outside this selected sector (OSS) (over portions of the island); and 3 at periods of variable winds. Bidleman and Olney (1975) concluded that toxaphene residues did not originate from Bermuda for the following reasons: (1) toxaphene had not been used on Bermuda for the prior 3 to 4 years; (2) mean concentrations for measurements OSS (0.81 ± 0.45 ng/cu m) and ISS (0.72 ± 0.09 ng/cu m) were similar; and (3) comparable residue levels were measured over the open ocean and over Bermuda. The authors concluded that the logical source of toxaphene was the U.S. cotton belt, 1200 km away.

Bidleman et al. (1976) monitored toxaphene residues at five North American sites for benchmark data. Sample collection followed the same procedure as that employed in the sampling of air over the Western North Atlantic. Table 7.5 describes the residues detected at the five sites. Toxaphene and other chlorinated hydrocarbons were never applied at the sites sampled. The lowest residue levels were measured at the northern sites (Rhode Island and Canada) and the highest levels at the southern sites (in Georgia and Arizona).

7.2.2 Residues in Water and Sediment

Although toxaphene has not been detected in any routine monitoring studies of U.S. surface waters (Weaver et al., 1965; Brown and Nishioka, 1967;

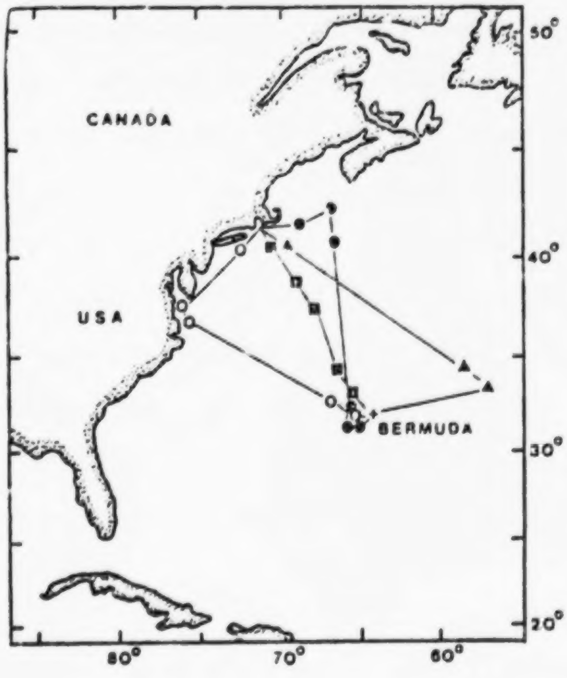
CANADA
USA
BERMUDA
50°
40°
30°
20°
80°
70°
60°

Figure 7.1. Atmospheric samples collected from the RV Trident, 1973 - 1974 (see Table 7.4 for cruise dates)
■, TR-137; ○, TR-145; ●, TR-152; ▲, TR-153.
Source: Bidleman and Olney, 1975.

Table 7.4. Concentrations of airborne toxaphene over the Western North Atlantic[a]

Location[b]	Dates	Toxaphene[c] (ng/cu m)
Bermuda 32°15'N 64°50'W	Mar. - Dec., 1973	< 0.02 - 3.3, 1.0 (11)[d]
Bermuda 32°15'N 64°50'W	Mar. - Oct., 1974	0.10 - 1.9, 0.57 (25)[d]
Sapelo Island, Georgia 31°15'N 81°05'W	May 20 - 26, 1975	1.7 - 5.2, 2.8 (6)[d]
Cruise TR-137	June 4 - 9, 1973	
33°20'N, 65°15'W		0.70
34°40'N, 66°15'W		1.2
37°40'N, 68°10'W		1.1
38°50'N, 69°15'W		0.52
40°30'N, 70°20'W		1.6
Cruise TR-145	Oct. 20 - 31, 1973	
32°00'N, 65°00'W		0.87
32°40'N, 67°20'W		0.57
36°40'N, 75°20'W		0.39
37°00'N, 76°00'W		0.77
37°20'N, 76°10'W		0.77
40°30'N, 72°40'W		0.65

---

[a] Source: Bidleman and Olney, 1975.
[b] Midpoint of the collection track.
[c] The mean blank corresponded to 0.03 ng/cu m for a 1,000 cu m sample.
[d] Range, mean and (number of samples).

Table 7.4 (continued)

Location[b]	Dates	Toxaphene[c] (ng/cu m)
Cruise TR-152	May 9 - 19, 1974	
31°20'N, 64°50'W		0.16
31°30'N, 65°20'W		< 0.1
32°00'N, 65°30'W		0.39
40°40'N, 66°30'W		< 0.06
41°30'N, 68°30'W		< 0.04
42°20'N, 66°40'W		0.05
Cruise TR-153	May 25 - 31, 1974	
33°40'N, 57°00'W		0.11
34°30'N, 58°40'W		0.58
40°30'N, 70°00'W		0.04

---

[a] Source: Bidleman and Olney, 1975.
[b] Midpoint of the collection track.
[c] The mean blank corresponded to 0.03 ng/cu m for a 1,000 cu m sample.
[d] Range, mean and (number of samples).

Table 7.5. Toxaphene residues in air samples at five North American sites[a]

Location and date	Number of samples	Range (ng/cu m)
Kingston, RI, 1975	6	0.04 - 0.4
Sapelo Island, GA, 1975	6	1.7 - 5.2
Organ Pipe Cactus National Park, AZ, 1974	6	2.7 - 7.0
Hays, KS, 1974	3	0.083 - 2.6
Northwest Territories, Canada, 1974	3	0.04 - 0.13

[a] Source: Adapted from Bidleman et al., 1976.

Lichtenberg et al., 1970; Manigold and Schulze, 1969; Schulze et al., 1973; Schafer et al., 1969; Mattraw, 1975), 1,2,3,4,5,7,7-heptachloronorbornene, which is claimed as a possible toxaphene component (Sanborn et al., 1976), has been found in New Orleans drinking water at 0.06 ppb (U.S. EPA, 1974). Since the compound is neither a terpene nor a likely metabolite, the claim that toxaphene is its source is in serious doubt. The routine monitoring studies have screened for residues by procedures which will detect the simple organochloride residues (e.g., DDT and its residues, dieldrin, heptachlor epoxide) at significantly lower concentrations than toxaphene (Section 2.6.1). Lichtenberg (1971) and Schulze et al. (1973) place the toxaphene lower detection limit at 0.5 to 1.0 ppb, whereas other organochlorides can be detected at concentrations about two orders of magnitude lower. While Lichtenberg (1971) points out that a lower toxaphene detection limit could be achieved if toxaphene were specifically sought, the routine monitoring studies are either vague about toxaphene's lower detection limit or confirm that it is significantly higher than the other organochloride residues.

Toxaphene has been detected and monitored in waters and sediments where its origin was known. These include surface and ground waters in agricultural areas where toxaphene was applied to crops; lakes where toxaphene was added for rough fish control; and surface waters surrounding a toxaphene manufacturing plant at Brunswick, Georgia.

The Flint Creek, Alabama watershed was monitored during the years 1959 to 1965 (Nicholson et al., 1964; Nicholson et al., 1966; Nicholson, 1969; Grzenda and Nicholson, 1965; Grzenda et al., 1964). Although the watershed drains a diversified agricultural area, the study identified small cotton farms as the major pesticide users (Nicholson et al., 1964).

Natural water from Flint Creek and potable water from the Hartselle Water Treatment Plant (which is drawn from Flint Creek) were sampled in pairs by carbon-chloroform extraction (Section 2.6.2). Table 7.6 compiles data on toxaphene application in the watershed drainage area and the residues in the raw and finished waters. Actual residue concentrations in the waters are probably higher than reported by a factor of two, since the average toxaphene recovery from spiked samples was less than 50 percent (Section 2.6.6). Toxaphene concentration remained below 1 ppb in finished and raw waters throughout the study period. Water treatment by the Hartselle plant apparently did not significantly reduce the toxaphene concentration (Cohen et al., 1961).

Toxaphene residues varied seasonally. The maximum level, which was observed in summer, corresponded to the time that maximum application was expected. After toxaphene usage in the area had decreased by approximately an order of magnitude (in 1963 to 1964), the residues in water rapidly dropped. Nicholson et al. (1964) could not explain why toxaphene residues increased during 1960 to 1961, although its application decreased by approximately one-third from 1959 to 1960.

The Flint Creek study also measured residues in biota (Section 4.0 and 5.0), soil (Section 7.2.3), and sediment. Grzenda and Nicholson (1965) reported that no toxaphene was detected in 58 samples of mud from eight sites in Flint Creek. Sediment samples were collected between June and December 1963. Table 7.7 summarizes the number of positive detections of toxaphene, DDT, and BHC residues in suspended sediments of raw water, in filtered, raw water, and in treatment plant sediments at the Hartselle Water Treatment Plant. Since they detected toxaphene less often on suspended solids than DDT,

Table 7.6. Toxaphene residues recovered from Flint Creek, Alabama and the Hartselle Water Treatment Plant[a]

Year	Technical toxaphene usage [thousands of kilograms (pounds)]	Toxaphene concentration (ppb)									
		Summer				Fall		Winter		Spring	
		Untreated water		Treated water		Untreated water	Treated water	Untreated water	Treated water	Untreated water	Treated water
		Mean	Range	Mean	Range	Mean	Mean	Mean	Mean	Mean	Mean
1959-60	63.3 (56.5)	0.105	0.010-0.260	0.105	0.040-0.280	0.029	0.079	0.030	0.046	0.049	0.052
1960-61	42.5 (37.9)	0.140	0.010-0.270	0.210	0.010-0.410	0.067	0.063	0.027	0.018	No sample	
1961-62	72.4 (64.6)	0.145	0.110-0.164	0.074	0.037-0.111	0.040	0.030	0.051	0.052	No sample	
1962-63	60.7 (72.0)	0.054	0.025-0.125	0.095	0.045-0.154	0.049	0.066	0.050	0.043	0.01	
1963-64	8.4 (7.5)			0.16[sic]	0.04-0.08		0.03		0.001	0.01	
1964-65	9.0 (8.0)			0.05	0.00-0.11		0.01		0.00	0.00	
1965	9.5 (8.5)			0.01	0.00-0.08		0.00				

[a] Source: Adapted from Nicholson et al., 1964, 1966.

Table 7.7. Comparison of insecticide recovery from sediment and water, Hartselle, Alabama Water Treatment Plant[a]

Sample source	No. sample	Percent positive for			
		DDT	DDE	Toxaphene	BHC
Sediment from treatment plant settling basis	45	71	64	18	22
Suspended sediment extracted from raw water by filtration prior to carbon filtration[b]	77	69	62	10	17
Carbon adsorption samples collected from water after removal by above filtration	77	13	12	31	74

[a] Source: Nicholson et al., 1966.

[b] A Cuno Micro-Klean filter that removed sedimentary particles larger than 25 microns was used. Smaller particles pass through to the carbon adsorption units.

they concluded that aquatic toxaphene residues are primarily dissolved rather than adsorbed onto particulates.

Bailey and Hannum (1967) monitored toxaphene in California surface waters and their sediments. Table 7.8 summarizes their measurements. Toxaphene concentrations were higher in sediments than in the water. They also noted that the pesticide concentrations generally were greater on the smaller size particles.

Johnston et al. (1967) discovered that toxaphene and other insecticide residues had contaminated an aquifer in the San Joaquin Valley, California. The contamination was detected in an experimental plot in which the movement of other insecticides was being examined. A plot, to which pesticides had not previously been applied, was subdivided into three blocks. The blocks were flooded and subsequent drainage from each block was analyzed. Although toxaphene had never been applied, it was detected in the drainage (Table 7.9). They concluded that its source was contaminated ground water. Johnston et al. (1967) also detected toxaphene residues in 13 of 66 analyses of other San Joaquin Valley tile drainage effluents (average 0.528 ppb and range 0.130 to 0.950 ppb) and in 60 of 61 analyses of surface effluent in Panoche Drain Water (average 2.009 ppb and range 0.100 to 7.900 ppb).

In their annual reports, the San Joaquin District, California Department of Water Resources (1968 to 1969), which monitored the area's water, detected toxaphene in 51 of 422 (12 percent) tile drainage effluents (0.02 to 0.50 ppb), in 216 of 447 (48 percent) of surface drains in Central Valley (0.04 to 71.00 ppb), in 88 of 712 (12 percent) of Central Valley surface waters (0.02 to 0.93 ppb), and in 8 of 200 (4 percent) of California bays and surface waters.

Table 7.8. Toxaphene concentrations in California surface waters and their sediments[a]

Sampling station	Concentration (micrograms per liter)					
	Surface Water			Sediment		
Streams and bays	Maximum	Minimum	Average	Maximum	Minimum	Average
Feather River at Nicolaus Bridge	---	---	---	---	---	---
American River at Sacramento	---	---	---	---	---	---
Sacramento River at Walnut Grove	0.4	0.03	0.10	130	5	57
Mokelumne River at Highway 99			0.04			
Little Connection Slough at Atherton Road			0.160			170
Middle River at Victoria Canal						
Delta Mendota Canal at Head	0.120	0.03	0.08			
San Joaquin River at Antioch	0.320	0.050	0.145			140
Suisun Bay at Martinez	0.090	0.050	0.063			
Napa River at Duttens Landing						
San Pablo Bay at Point San Pablo			0.08			110
San Francisco Bay at Berkley Pier	0.23	0.03	0.13			
San Francisco Bay at Treasure Island						
San Francisco Bay at San Mateo Bridge			0.26			110

[a] Source: Bailey and Hannum, 1967.

Table 7.8 (continued)

Sampling station Streams and bays	Concentration (micrograms per liter)					
	Surface Water			Sediment		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Golden Gate Bridge at Fort Point						
San Joaquin River at Vernailes	0.93	0.02	0.26			
San Joaquin River at Fremont Ford	0.46	0.04	0.13			
Salton Sea near North Shore	0.40	0.05	0.14			
Alamo River	0.65	0.30	0.47			
All American Canal at Alamo River	0.08	0.04	0.06			
Agricultural Drainage						
Reclamation District No. 108 Drain						210
Colusa Basin Drain			0.230			
Staten Island Drain						110
Roberts Island Drain at Whiskey Slough						380
Panoche Drain	5.50	0.100	1.467			
Salt Slough	0.440	0.040	0.169			

Table 7.9. Toxaphene residues in tile effluent drainage from a plot not previously treated with toxaphene[a]

	Toxaphene concentration (ppt)		
	Block A	Block B	Block C
First flooding	50	500	100
Second flooding	175	50	0
Third flooding	550	0	0

[a] Source: Johnston et al., 1967.

Barthel et al. (1969) examined organochloride residues in sediments of the lower Mississippi River and its tributaries during 1966. Toxaphene was detected at few of the sampling sites; Table 7.10 summarizes sites and concentrations.

The University of Georgia Marine Institute (Reimold, 1974; Reimold and Durant, 1972 a, b, 1974; Durant and Reimold, 1972) has monitored toxaphene contamination in surface waters, sediment, and biota of waters receiving the effluent of the Hercules, Inc. plant located on Terry Creek, Brunswick, Georgia. This plant is the largest producer of toxaphene in the U.S. Figure 7.2 describes the average monthly toxaphene concentration in the plant's effluent from 1970 to 1974. It has decreased from a high of 2332 ppb in August 1970 to a low of 6.4 ppb in June 1974. Dye experiments have shown that the effluent is diluted by a factor of 10 after it reaches Terry Creek (Reimold, 1974). The Institute (Reimold and Durant, 1972 a, b; Durant and Reimold, 1972) analyzed sediment at three locations downstream of the plant outfall. Samples were collected prior to a dredging operation in June 1971 at sites downstream: 0.2 miles from the outfall at a location 50 yards from its intersection with another creek; 0.8 miles from the plant outfall; and 1.4 miles from the plant outfall and 50 yards from the end of Terry Creek (junction with Back River). Table 7.11 summarizes toxaphene concentration in sediment cores to 80 cm deep. Reimold and Durant (1972 b) measured 32.56 ppm as the average toxaphene concentration within Terry Creek Marsh. The highest residue concentration measured in the surrounding water was 0.015 ppm.

Mattraw (1975) screened for organochloride residues in soil, water, and its sediment in southern Florida from 1968 to 1972. The area includes undeveloped

Table 7.10. Toxaphene residues in sediments of the lower Mississippi River and its tributaries in 1966[a]

River	Site	Concentration of toxaphene/Strobane[b] in oven-dried sample (ppm)
Mississippi River	West Memphis, Arkansas	
	River mile 727.7	
	Site UL-2	0.4
	Site UL-7	0.1
Sunflower River	Coahoma County, Mississippi	
	Site M	11.19
	Site M	11.93
	Site D	8.85
	Site D	10.87
Sunflower River	Sunflower County, Mississippi	
	Site U	2.45
	Site D	6.57
Ditch 24, Sunflower River	Sunflower County, Mississippi	13.18
Jones Bayou	Bolivar County, Mississippi	
	Site U	4.52
	Site D	3.91

[a] Source: Barthel et al., 1969.

[b] Strobane is a mixture of chlorinated diterpenes which is produced by chlorinating α-pinene. It closely resembles toxaphene (see Section 2.4.2).

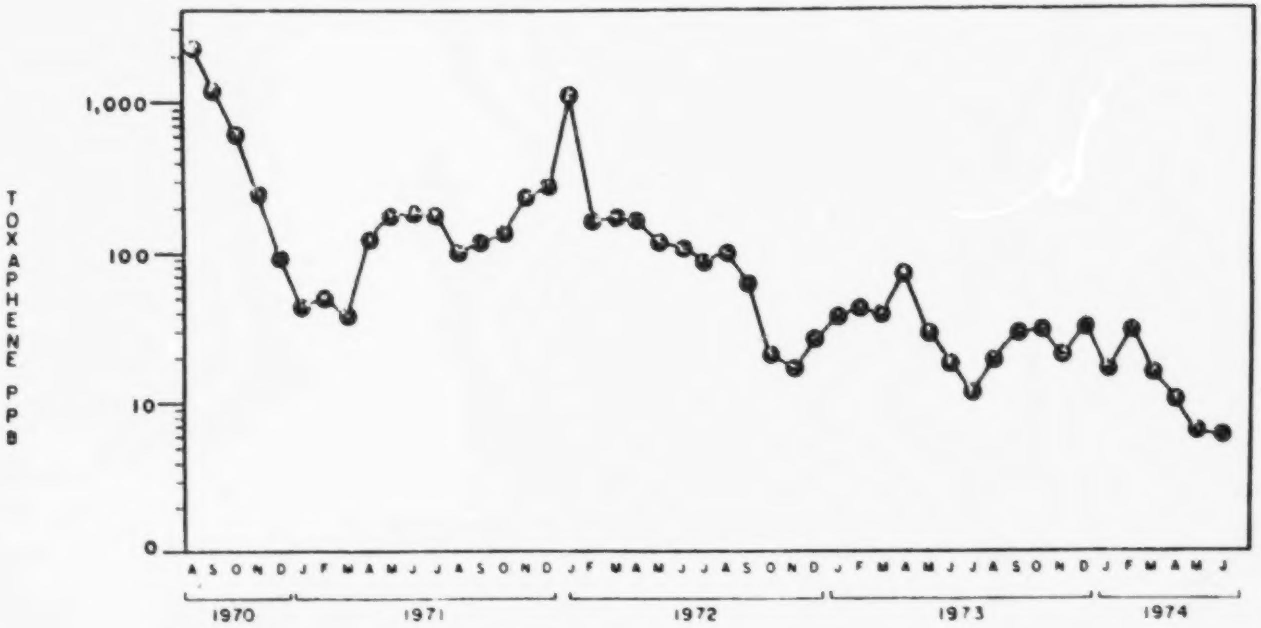

Figure 7.2. Monthly average of apparent toxaphene concentration of manufacturing plant effluent, 1970 - 1974. Source: Reimold, 1974.

Table 7.11. Toxaphene concentrations in three sediment cores, by 10 cm increments, collected from Terry Creek, Brunswick, Georgia, June 10, 1971[a]

Sampling location	Residues (ppm) and content of sediment							
	Surf. to 10 cm	10 - 20 cm	20 - 30 cm	30 - 40 cm	40 - 50 cm	50 - 60 cm	60 - 70 cm	70 - 80 cm
Site C[b]	1,858.3 mud and some chips	1,340.5 mud and few chips	1,324.0 mud	1,367.2 mud	1,236.7 mud	433.6 mud	68.5 mud	83.2 mud
Site D[c]	111.85 mud and few chips	615.64 mud and many chips	16.04 mud	17.46 mud	5.42 mud	3.4 mud	2.88 mud	No sample
Site E[d]	35.5 mud	35.47 mud	21.9 mud	70.65 mud	79.8 mud	21.0 mud	18.5 mud	5.27 mud

[a] Source: Durant and Reimold, 1972 b.
[b] North shore of Terry Creek, 50 yd from junction with Dupree Creek and .2 mile from toxaphene plant outfall.
[c] South shore of Terry Creek, .8 mile from the plant outfall and one-half the distance from the outfall to Back River.
[d] South shore of Terry Creek, 1.4 miles from the plant outfall and 50 yd west of Back River.

lands, agricultural lands, and coastal urban areas. Toxaphene residues were not detected in 146 samples of surface water. Although Mattraw (1975) defines the limit of detection for water samples at 0.005 ppb for all organochlorides, the limit is expected to be higher for toxaphene (Sections 2.6.1 and 7.2.3). Toxaphene was detected (claimed lower detection limit of 0.05 ppb) in 3.2 percent of sediment samples and 4.2 percent of cropland soil.

Keith (1966) examined toxaphene and other chlorinated hydrocarbon insecticides in the sediments of Tule Lake and Lower Klamath Lake National Wildlife Refuge in northeastern California. Both lakes are impoundments which are used for agricultural purposes. While the lakes were not directly treated with toxaphene, their waters were used for irrigation and subsequent agricultural drainage was returned. Keith (1966) estimated that waters of the refuge were used from five to seven times for irrigation. Sediment toxaphene concentrations in these lakes ranged from 0.0 to 0.2 ppm for the years 1960 and 1961.

Terriere et al. (1966) monitored residues in two Oregon lakes to which toxaphene was applied as a piscicide. Table 7.12 describes the residue concentrations in the water and bottom sediment. Miller Lake is deep and biologically sparse, while Davis Lake is shallow and rich in aquatic life.

Kallman et al. (1962) applied 0.05 ppm of toxaphene to Clayton Lake, New Mexico in three stages. It was added as 0.7 kg per liter (emulsifiable concentrate) for rough fish control. Figure 7.3 describes the additions and subsequent monitoring of aqueous toxaphene at the 3.05 meter level. The solid columns denote amounts in water from the windward shore and the barred columns from the lee shore. Vertical arrows and their numbers denote the times at which applications were made and the amounts applied. They found

Table 7.12. Toxaphene residues in Miller Lake and Davis Lake, Oregon[a]

	Toxaphene concentration (ppb)							
	Miller Lake				Davis Lake			
(Year)	1958	1962	1963	1964	1961	1962	1963	1964
Toxaphene application rate	40	--	--	--	88	--	--	--
Aqueous residue	--	2.10 (0.7-3.1)	1.2 (0.7-1.6)	0.8 (0.7-1.1)	--	0.63 (0.5-0.9)	0.41 (0.3-0.6)	< 0.2
Bottom mud	--	750 (390-1430)	391 (330-13800)	--	--	650 (100-1000)	800 (100-3100)	--

[a] Terriere et al., 1966.

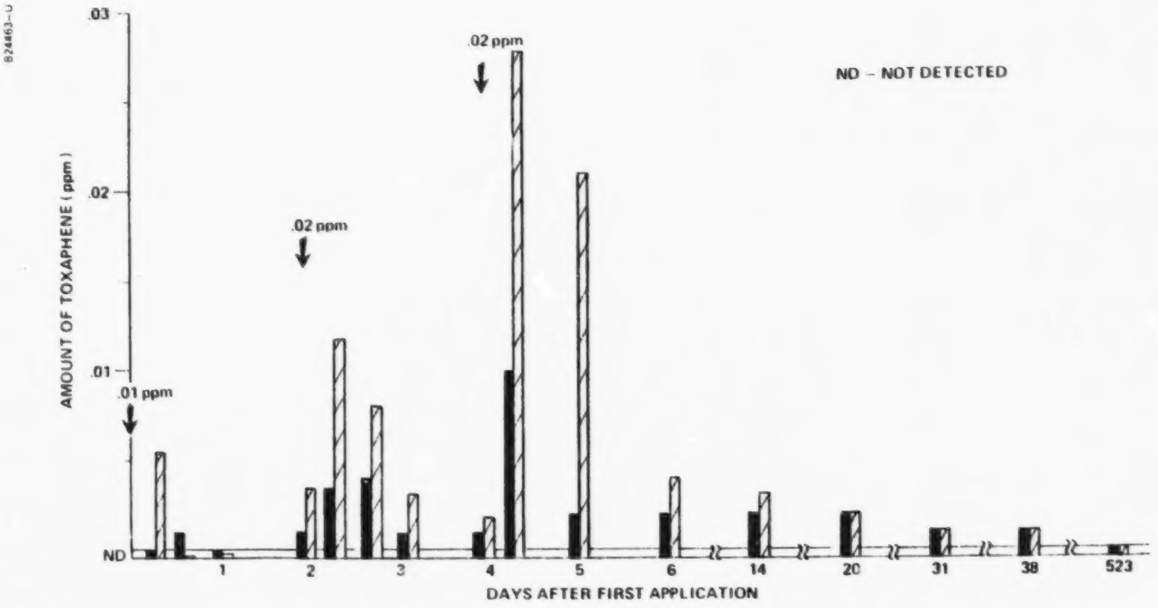

Figure 7.3. Amount of toxaphene measured in Clayton Lake (New Mexico) water from the 3.05 meter level before and after applications of toxaphene. Source: Kallman et al., 1962.

a maximum toxaphene concentration in sediment of 0.15 ppm, which was measured six days after the first addition.

Johnson et al. (1966) examined toxaphene residues in Wisconsin lakes to which toxaphene was applied for fish control. Table 7.13 describes the toxaphene application and residues found in 1965.

7.2.3 Residues in Soil

Stevens et al. (1970) examined insecticide application and soil residues at 51 sites from 1965 to 1971. They found that 17 sites regularly applied pesticide, 16 sites applied pesticide at least once but that application was not regular, and 18 sites never had applied a pesticide. No toxaphene residues were detected at the sites of limited or no pesticide use. Table 7.14 summarizes the known application rates and residue levels. Although only 5 sites reported that toxaphene had been applied, toxaphene residues were detected at 7 sites. The 2 sites with residues but no history of application were Tule Lake, California, and Wenatchee, Washington. The regular pesticide use croplands were classified into three groups: vegetable and/or cotton; tree fruit; and small grain and root crops. Toxaphene was detected at 60 percent of the fields in the vegetable and/or cotton croplands (range 0.66 to 9.38 mg/kg), at one orchard (7.72 mg/kg), and at 12 percent of the small grain and root croplands (range 0.11 to 2.01 mg/kg).

EPA's National Soil Monitoring Program (NSMP) has monitored soils of croplands, non-croplands, and urban areas since 1967. Wiersma et al. (1972 b) examined cropland in 43 states and non-cropland in 11 states during fiscal year 1969. They detected toxaphene in only 1 of 199 non-cropland soils (concentration 0.52 ppm). Toxaphene was detected in 4.2 percent (73

Table 7.13. Toxaphene in Wisconsin lakes, 1965[a]

		Concentration (ppb)				
Lake	Year of treatment	Treatment rate	Water[b]	Suspended matter	Aquatic plants	Sediment
Little Green	1956	100	1	40	---	20
Emily	1959	100	4	20	400	200
Kusel	1960	100	3	200	70	400
Marl	1960	100	3	9	80	1000
Big Twin	1963	100 & 50	2	20	40	800
Wilson	1964 May 1965	2.5 - 3.5 5 Epilimnion only	4	80	50	500
Round	1964 & 1965	5 + 5	2	200	80	600
Comstock (surface)			20	100	50	1000
6.5 meters	June, 1965[c]	100	4	500	--	----

---

[a] Source: Johnson et al., 1966.
[b] Remaining in water after filtration through Whatman GF/A glass filters.
[c] Comstock Lake was treated 14 days prior to sampling.

Table 7.14. Toxaphene monitoring in U.S. croplands: application rates and soil residues[a]

Location	Toxaphene applied		Toxaphene residue	
	kg/ha (lbs/acre)	Year	mg/kg	Year
Kern County, California	---	---	---	---
Lower Rio Grande Valley, California				
Field 1	18.3 (16.2)	1956-1964	2.90	Oct. 1966
	3.4 (3.0)	1965		
	2.3 (2.0)	1966		
Field 2	53.0 (47.0)	1958-1964	1.98	Oct. 1966
	7.9 (7.0)	1965		
	1.4 (1.25)	1966		
Field 3	38.4 (34.0)[b]	1958-1964	1.77	Oct. 1966
	10.2 (9.0)	1965		
	1.1 (1.0)	1966		
Field 4	33.0 (29.25)	1955-1964	2.01	Oct. 1966
Field 5	44.2 (39.16)	1956-1964	2.43	Oct. 1966
Monmouth County, New Jersey	---	---	---	---
Dade County, Florida				
Field 1	4.5 (4.0)	1965	1.21	Mar. 1968
Field 2	9.0 (8.0)	1958-1964	2.64	Mar. 1968
	4.5 (4.0)	1965		
	2.3 (2.0)	1966		
Field 3	4.5 (4.0)	1965	0.66	Mar. 1968
Field 4	2.5 (2.2)	1962-1964	4.42	Nov. 1966
	10.2 (9.0)	1965	4.14	Mar. 1968
Field 5	35.6 (31.59)	1962-1964	9.38	Nov. 1966
	22.4 (19.90)	1965	7.00	Mar. 1968
Eastern South Carolina				
Field 1	3.4 (3.0)	1952-1964	2.99	Aug. 1966
Field 2			1.05	Aug. 1966
Field 3	16.9 (15.0)	1956-1964		
	3.4 (3.0)	1966	5.64	Aug. 1966
Field 4	53.0 (47.0)	1957-1964	0.99	Aug. 1966
	10.2 (9.0)	1965		
	20.3 (18.0)	1966		
Field 5	42.9 (38.0)	1956-1964	2.04	Aug. 1966

[a] Stevens et al., 1970.

[b] 1.9 kg/ha (1.70 lb/acre) Strobane was also applied (see Table 7.10, footnote b).

Table 7.14. (continued)

Location	Toxaphene applied		Toxaphene residue	
	kg/ha (lb/acre)	Year	mg/kg	Year
Wenatchee, Washington Orchards 1, 2, 3, 4	---	---	---	---
Orchard 5	---	1959-1967	7.72	Oct. 1967
Adams County, Pennsylvania	---	---	---	---
Berrien County, Michigan	---	---	---	---
Western North Carolina	---	---	---	---
Yuma, Arizona	---	---	---	---
Central, Georgia	---	---	---	---
Quincy and Moses Lake, Washington Fields 1 and 5	---	--	---	---
Field 2	3.4 (3.0)	1963-1964	---	---
Field 3	1.7 (1.5)	1965	---	---
Field 4	30.3 (27.0)	1956-1964	0.95	Oct. 1967
Tulelake, California Field 1	---[b]	---	0.58	Sept. 1967
Fields 2 - 5	---	---	---	---
Weld County, Colorado Fields 1, 2, 4	---	---	---	---
Field 3	Unknown	1966	0.78	Oct. 1966
	---	---	2.01	Sept. 1967
Field 5	---	---	0.11	Sept. 1967
Urbana, Illinois	---	---	---	---
Western Iowa	---	---	---	---
Eastern Virginia	---	---	---	---

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[a] Stevens et al., 1970.
[b] 1.9 kg/ha (1.70 lb/acre) Strobane was also applied (see Table 7.10, footnote b).

of 1729) of cropland sites with a concentration of 0.10 to 11.72 ppm. The mean toxaphene concentration for all cropland sites was 0.07 ppm. Wiersma et al. (1971) sampled 242 cropland and 117 non-cropland soils in six states in 1967. Toxaphene residues were not detected in non-cropland soil. Results for the cropland soils are presented in Table 7.15. Residues were only found in two states; Georgia (10 sites) and Idaho (1 site).

Crockett et al. (1974) published data for toxaphene application and soil residues at 1506 cropland sites in 35 states, which were monitored during fiscal year 1970. Toxaphene was reportedly used at 33 (2.45 percent of sites) sites and applied at an arithmetic mean rate of 0.023 kg/ha. Table 7.16 summarizes the results by state. Toxaphene was detected at 27 sites: 13 in California; 5 in Mississippi; 3 in Georgia; 2 in Louisiana and South Carolina; and one in Florida, Oklahoma, and Kentucky. Toxaphene residues at detected sites ranged from 0.79 to 8.75 ppm. Residues are listed by crop in Table 7.17. Toxaphene was most often detected at sites classified as cotton or irrigated lands and the residues found at those sites had the highest concentration: arithmetic mean of 0.68 ppm for irrigated land and 0.32 ppm for cotton farming.

Wiersma et al. (1972 a) measured toxaphene and other pesticide residues in soil from eight cities in 1969. Table 7.18 summarizes the toxaphene data. In each city, fifty random sites were sampled. Toxaphene residue was detected at only four sites: two sites in Miami and one site each in Houston and Manhattan (Kansas). While toxaphene residues never exceeded 4 percent of the sites in any city, DDT residues were detected at 40 percent (Houston) to 100 percent (Miami) of sampled urban sites.

Table 7.15. Cropland soil monitoring for toxaphene in six states, 1967[a]

State	Sites sampled	Sample sites treated with toxaphene (percent)	Sites with toxaphene residues (percent)	Toxaphene residues (ppm)
Georgia	30	13.3	33.3	0.55
Idaho	33		3.1	0.44
Maine	8		0	
Nebraska	107		0	
Virginia	19	5.3	0	
Washington	45		0	
Overall	242		4.6	0.13

[a] Source: Wiersma et al., 1971.

Table 7.16. Toxaphene in cropland soil by state, fiscal year 1970[a]

State	Times detected	Sites sampled	Toxaphene concentration (ppm) Arithmetic mean[b]	Toxaphene concentration (ppm) Range of detected residues
All sites	27	1506	0.06	0.79-8.75
Alabama	c	21		
Arkansas	c	47		
California	13	65	0.61	0.79-7.63
Florida	1	17	0.34	5.71
Georgia	3	28	0.17	1.21-2.27
Illinois	c	140		
Indiana	c	78		
Iowa	c	150		
Kentucky	1	30	0.03	0.89
Louisiana	1	26	0.20	5.32
Michigan	c	54		
Mid-Atlantic states[d]	c	19		
Minnesota	c	120		
Mississippi	5	29	0.73	2.79-8.75
Missouri	c	81		
Nebraska	c	106		
New England States[e]	c	20		
New York	c	38		
North Carolina	c	30		
Ohio	c	69		
Oklahoma	1	65	0.02	1.60
Pennsylvania	c	32		
South Carolina	2	17	0.34	2.44-3.34
South Dakota	c	106		
Tennessee	c	25		
Virginia, West Virginia	c	26		
Wisconsin	c	67		

[a] Source: Adapted from Crockett et al., 1974.

[b] For all sites samples.

[c] None reported.

[d] Mid-Atlantic states include Maryland, Delaware, and New Jersey.

[e] New England states include Maine, New Hampshire, Massachusetts, Rhode Island, and Connecticut.

Table 7.17. Toxaphene residue in cropland soil by cropping region, fiscal year 1970[a]

Crop	Times detected (percent)	Sites analyzed	Arithmetic mean concentration[b] (ppm)
Corn	N.D.[c]	713	N.D.
Cotton	8.91	101	0.32
Cotton and general farming	3.42	117	0.09
General farming	3.40	147	0.07
Hay and general farming	N.D.	184	N.D.
Irrigated land	20.51	39	0.68
Small grains	N.D.	105	N.D.
Vegetables	N.D.	72	N.D.
Vegetables and fruit	2.38	42	0.14

[a] Source: Adapted from Crockett et al., 1974.
[b] For all sites analyzed.
[c] Not detected.

Table 7.18. Toxaphene residues in soil from eight cities, 1969[a]

City	Range	Average[b]	Percent[c] positive sites
Bakersfield, CA	N.D.[d]		
Camden, NJ	N.D.		
Houston, TX	0.11[e]	<0.01	2.0
Manhattan, KS	12.07[e]	0.24	2.0
Miami, FL	14.79 - 52.73	1.34	4.0
Milwaukee, WI	N.D.		
Salt Lake City, UT	N.D.		
Waterbury, CT	N.D.		

[a] Source: Wiersma et al., 1972 a.
[b] Based on total number of samples (49).
[c] Percent based on number of sites with residues greater than or equal to the sensitivity limits.
[d] Not detected.
[e] One value.

The National Soil Monitoring Program examined cropland soils of the corn belt states (Carey et al., 1973), 10 onion growing states (Wiersma et al., 1972 c), and 9 sweet potato farming states (Sand et al., 1972). No toxaphene residues were detected from sweet potato or corn growing soils, but residues were found in 12.7 percent of the 76 onion growing sites. While toxaphene residues averaged 0.55 ppm at the onion growing sites, at positive detection sites they ranged from 2.19 to 7.77 ppm.

Grzenda and Nicholson (1965) examined toxaphene residues in soil samples from fields surrounding Flint Creek, Alabama. Their work was part of an intensive study of toxaphene, DDT, and BHC use and residues (Section 7.2.2). They detected toxaphene in 58 percent of the soils collected from 33 cotton fields. Mean concentration (33 fields) was 0.41 ng/kg. Toxaphene concentrations (positive sites) ranged from 0.16 to 1.60 ng/kg.

Mullins and his coworkers (1971) examined pesticide applications and residues in major agricultural areas of Colorado. Of the 50 sites evaluated, 9 reported toxaphene applications with an average amount applied of 3.14 ppm. Toxaphene (at 1.00 ppm) was detected at only one site.

7.3 ENVIRONMENTAL FATE

7.3.1 Mobility and Persistence in Air

The relatively high vapor pressure of toxaphene (0.17 to 0.4 mm Hg) compared to other chlorinated hydrocarbon pesticides (0.000001 to 0.000000001 mm Hg) suggests that atmospheric processes will have considerable impact upon the environmental fate of toxaphene. Toxaphene has been detected in air in concentrations as high as 2520 ng/cu m, the concentration varying depending upon location (near or remote from crop spraying) and time of year (high during summer months when pesticides are being used most (Section 7.2.1).

The several monitoring studies that have detected toxaphene in air all illustrate the ability of toxaphene to be transported by atmospheric currents. This is best demonstrated by the work of Bidleman and coworkers (Bidleman and Olney, 1975; Bidleman et al., 1976) who detected toxaphene 1200 km out to sea. When the trajectories of the samples were examined, it was found that samples with North American air sources differed by no more than a factor of two from the samples whose trajectories did not pass over the continent. The authors indicated that these results suggest that toxaphene has a sufficiently long lifetime in the atmosphere to become well-mixed in the North Atlantic atmosphere. However, the higher concentrations detected when the air passed over North America suggests that a likely source of airborne toxaphene is from its use in the southern U.S. cotton growing areas. The mean levels of toxaphene detected in the North Atlantic Ocean air are equal to or twice those of PCB's and ten times higher than those of other pesticides, such as DDT.

The form in which toxaphene is present in the atmosphere (particulate or gaseous) is unknown. Bidleman and Olney (1975) found that less than 5 percent of the total toxaphene was found on glass fiber filters (collects 98 percent of particles $\geq$ 0.015 micrometer) which suggests that toxaphene may be mostly gaseous. However, their air flow rates (0.48 to 0.76 cu m/min) in the sampler might allow evaporation from the particulate matter.

The stability of toxaphene in the air has not been studied. For example, no vapor phase photolysis studies are available. However, comparison of gas chromatographs of standard toxaphene and an air sample obtained in Bermuda (Bidleman and Olney, 1975) show some slight changes in peak heights and retention times. These differences might be explained by selective volatilization of the more than 175 compounds included in toxaphene. Seiber et al. (1975)

found that a toxaphene air sample gathered above a cotton field and analyzed by gas chromatography showed a substantial enrichment of the more volatile components. Enrichment of the less volatile components was found on leaf surfaces.

7.3.2 Mobility and Persistence in Water

Although toxaphene is a widely used insecticide, few laboratory studies have been conducted on its environmental fate in aqueous systems. Most investigations relevant to the environmental behavior of toxaphene in water have been field studies, mostly monitoring studies of water systems near cotton fields or lakes that have been treated with toxaphene for rough fish control.

Toxaphene has not been detected in routine ambient water monitoring investigations, which might suggest a lack of persistence of toxaphene in water systems. An alternative explanation is that the analytical methods used for screening chlorinated hydrocarbon pesticides are not as sensitive for toxaphene as the methods are for less chemically complex compounds.

Analysis of water samples which contain toxaphene (Section 7.2.2) provides some insight into the mode of transport. Nicholson et al. (1966) detected toxaphene less often than DDT in suspended solids, even though the percentage of samples containing DDT in the water by carbon chloroform extraction was lower than the percentage containing toxaphene. These authors concluded that aquatic toxaphene residues are primarily dissolved rather than adsorbed onto particulates and are not removed by conventional water treatment plants (Cohen et al., 1961). This suggestion is supported by the work of Grzenda and Nicholson (1965) who found amounts of toxaphene in river water varying from 30 to 140 ppt in Flint Creek, Alabama, but no toxaphene in river bottom sediment.

On the other hand, Bailey and Hannum (1967) found higher concentrations of toxaphene in sediment than in water and the concentration was greater on smaller sized particles.

Toxaphene residues in water may be removed by a variety of mechanisms including biodegradation, hydrolysis, photodecomposition, evaporation, or codistillation, and adsorption to sediment or suspended particulates (followed by settling to the sediment). Studies conducted on lakes where toxaphene was used for rough fish control provide considerable information on the importance of the above mechanisms (Johnson et al., 1966; Kallman et al., 1962; Terriere et al., 1966; Veith and Lee, 1971; and Hughes, 1970). Toxaphene was used for rough fish control (piscicide) because it has the greatest fish toxicity of any chlorinated hydrocarbon, except endrin, and has a lower acute human toxicity than endrin, dieldrin, aldrin, or rotenone (Johnson et al., 1966). In addition, toxaphene's lower cost facilitated its use as a piscicide.

Prior to the use of modern analytical techniques, a number of studies which were based upon bioassays (fish kills) determined that a number of factors affected the rate of detoxification of toxaphene treated lakes. A variety of parameters have been suggested including sunlight, pH, temperature, dissolved oxygen concentration, alkalinity, hardness, concentration applied, turbidity, and presence of bacteria (Johnson et al., 1966; Veith and Lee, 1971). Studies using gas chromatographic methods could find little correlation between these factors. Johnson et al. (1966) studied eight Wisconsin lakes, almost all of which were classified as hard water, alkaline, shallow, eutrophic lakes with large amounts of plankton and higher plants in near shore areas. The lakes were treated with 0.1 ppm of toxaphene 3 to 9 years prior to sampling and contained 1 to 4 micrograms/liter of toxaphene in water, 0.2 to 1 ppm in sediments, and 0.05 to 0.4 ppm in plants. Although these residues were detected, the authors concluded that shallow eutrophic lakes detoxify rapidly. This finding is consistent with the results of Terriere et al. (1966) who studied two toxaphene treated Oregon lakes, one a deep and biologically sparse lake and the other a shallow and biologically rich lake. With the shallow lake, detoxification occurred in one year (concentration in water 0.6 microgram/liter), similar to the rapid detoxification observed by Johnson et al., (1966). However, with the deep lake the water concentration remained at 1.2 micrograms/liter after five years.

Veith and Lee (1971) concluded that toxaphene adsorption to sediment is a major detoxification mechanism which is consistent with the above conclusion that treated lakes which are stratified (deep lakes) remain toxic to fish longer than well-mixed, shallow lakes. They analyzed three lakes in Wisconsin that had been treated with toxaphene and also conducted a desorption study with toxaphene on sediment. The analysis of lake samples by gas chromatography with electron capture detection demonstrated that the concentrations in water rapidly decreased while the concentrations in sediment increased to a maximum and then decreased by a factor of 2 every 20 days. The authors noted that this rate of loss from sediment greatly differs from the 11 year half-life in soil reported by Nash and Woolson (1967). Analysis of various sediment sections (0 to 5 cm, 5 to 10 cm, 10 to 15 cm) showed that the concentration of toxaphene was much higher in the top layer. By measuring the time between treatment and the first detection of toxaphene in sediment, Veigh and Lee (1971) calculated the rate of vertical transport. For the three lakes, the values varied from 0.4 to 1.1 cm/day.

The desorption study of Veith and Lee (1971) consisted of placing various amounts of Ottman Lake sediments which contained 2.0 micrograms of toxaphene per gram (dry weight) in flasks containing filtered Ottman Lake water. The flasks were maintained at 23 to 25 C for 10 days with mixing four times daily. At 10 days the samples were filtered (glass fiber) and both water and sediment were analyzed. The toxaphene concentration remained below the detectable limit (1 microgram/liter) in water and was essentially the same as the starting concentration in sediment, thus suggesting strong adsorption between toxaphene and the sediment.

For each of the three lakes, Veith and Lee (1971) calculated the percentage of the applied toxaphene that was in the sediments at the time of maximum concentration. These percentages were 47.3 percent for Fox Lake at 51 days after treatment; 46.3 percent for Ottman Lake at 191 days; and 13.5 percent for Silver Lake at 275 days. Taking into account the low concentration in water (< 1 microgram/liter), the authors concluded that "greater than 50 percent of the toxaphene applied may have been lost during application, lost through evaporation and/or codistillation with water vapor, and degraded by microbial activity." As is the case now and was noted then by the authors, the significance of these additional processes has not been evaluated. However, some information on these processes is available.

Monitoring data of the air above the surface of toxaphene treated lakes or laboratory studies of the rate of evaporation of toxaphene from water are not available. However, Mackay and Leinonen (1975) have modified some work of Liss and Slater (1974) in order to provide a method for calculating the half-life of evaporation of low-solubility compounds from water bodies to the atmosphere using the vapor pressure, water solubility, and molecular weight of a compound. Using the available data for toxaphene (water solubility = .037 mg/liter; vapor pressure = 0.17 mm Hg; average molecular weight = 414), the calculated half-life for toxaphene evaporation from water is 10.4 hours. The rate of evaporation for each component of the toxaphene mixture will be different, and it is likely that changes in the gas chromatographic fingerprint could be attributed to selective evaporation (Seiber et al., 1975).

Few well controlled studies of the degradability of toxaphene in water systems have been conducted. Hughes (1970) reviewed a study by Mitchum (1963) where 1.0 mg/liter toxaphene was placed in some filtered, natural waters of Wyoming and held in the dark at temperatures of 26 C, 18 C, and 7 C for 521 days. Analysis of the water by paper chromatograph was undertaken every three months. Extrapolation of the toxaphene concentration in water vs. time curve provided estimated times for complete breakdown (645 days for 26 C; 2,680 days for 7 C). Interpretation of the data is difficult because no particulate matter is present (adsorption to particulates may affect rate of degradation). The presence of microorganisms in filtered water is unclear, and the initial concentration of toxaphene exceeds the water solubility of toxaphene (some loss might be due to precipitation [Hughes, 1970]).

Wolfe et al. (1976) have conducted some screening studies of hydrolysis and photolysis of toxaphene in aqueous media. They placed toxaphene in air-saturated dilute acidic and alkaline medium at 65 C. After two days they detected no change in the gas chromatographic fingerprint. In another study, they concluded that the photolysis of toxaphene under sunlight is a very slow process in pure water and that photochemically-generated singlet oxygen does not readily oxidize toxaphene. Sanborn et al. (1976) studied the fate of radiolabelled

(C-14 at the 8 carbon) and unlabelled toxaphene in a terrestrial-aquatic model ecosystem. Gas chromatography with electron capture detection was used with the unlabelled material for comparison. The results (Table 7.19) show that toxaphene is stable enough to be bioaccumulated in substantial quantities in a variety of aquatic organisms. On the other hand, the results also indicate some breakdown to new compounds or unextractables. However, in terms of the degradability of toxaphene in natural water systems, the results are difficult to interpret. The normal operation of the terrestrial-aquatic model ecosystem uses sand, rather than sediment, which most likely will have considerable impact on the rate of breakdown as well as the degree of bioaccumulation.

Investigators monitoring water and sediment samples have long noticed a difference between the gas chromatography fingerprint of residue toxaphene and standard toxaphene. Johnson et al. (1966) detected 1 to 4 micrograms/liter of toxaphene in eight treated Wisconsin lakes. This concentration of standard toxaphene is used to kill small fish, yet the residue concentration was not toxic to fish. The authors found differences in the gas chromatographic fingerprint and suggested that components of toxaphene, especially the ones responsible for fish toxicity, degrade at different rates. Similar results were found by Terriere et al. (1966) and Hughes (1970). Hughes et al. (1970) concluded that selective degradation of components does occur to a limited extent at a slow rate in the aquatic environment.

Hughes (1970) is the only researcher who has attempted to characterize the alterations that occur. Typical fingerprint alterations for toxaphene residues from lake water or sediment are illustrated in Figures 7.4 and 7.5. In general, the gas chromatographic changes consist of the diminution of

Table 7.19. Toxaphene in a terrestrial-aquatic model ecosystem[a]

			Toxaphene[b] equivalents (ppm)			
	R_f[c]	Water	Oedogonium (alga)	Phyea (snail)	Culex (mosquito)	Gambusia (fish)
Total ^{14}C		0.04441	13.2941	17.6198	2.2570	10.3977
Toxaphene	0.70	0.00159	10.9743	15.2639	1.4147	6.7523
I[d]	0.57	0.00106	1.7535	1.8360	0.2159	2.4923
II	0.51	0.00076	-------	0.2961	------	0.5022
III	0.45	0.00099	0.3589	0.0863	------	0.3161
IV	0.34	0.00164	0.1130	0.0585	trace	0.1487
V (strip)		0.00429	--- --	-------	0.4042	------
VI	0.03	0.00078	-------	-------	------	0.0187
Origin	0.00	0.02002	0.0944	0.0211	0.2022	0.1674
Unextractable		0.01328	2.2156	1.1153	1.1245	4.2264

[a] Source: Sanborn et al., 1976.
[b] $C_{10}H_{10}Cl_8$ (67 - 69% chlorinated camphene) 8 - ^{14}C
[c] Silica Gel GF 254, Skellysolve B (bp 68°C): diethyl ether: acetone 80:20:10, by volume.
[d] Roman numerals - chemical structure unknown.

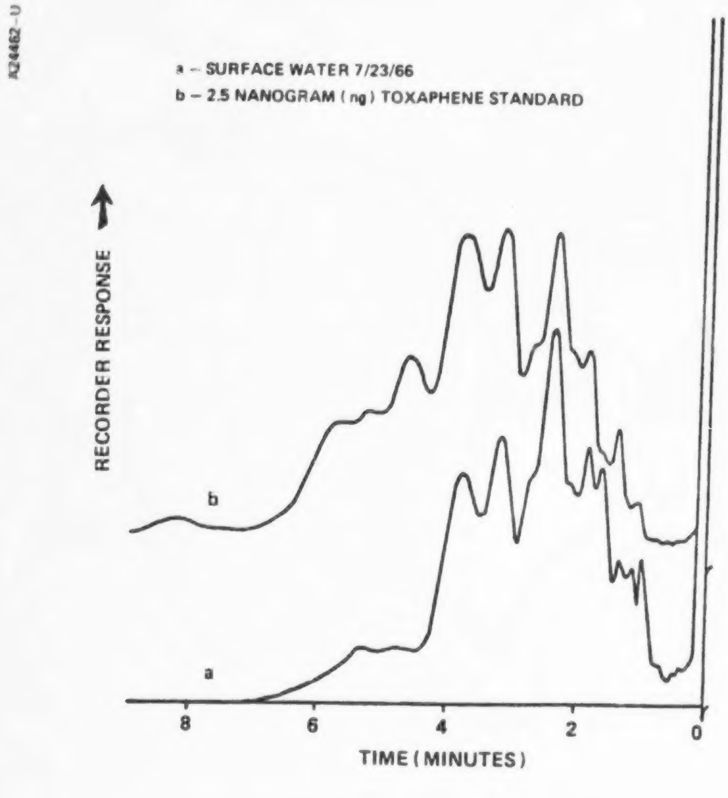

Figure 7.4. Gas chromatograms of standard toxaphene and toxaphene extracted from Comstock Lake Water. Source: Hughes, 1970.

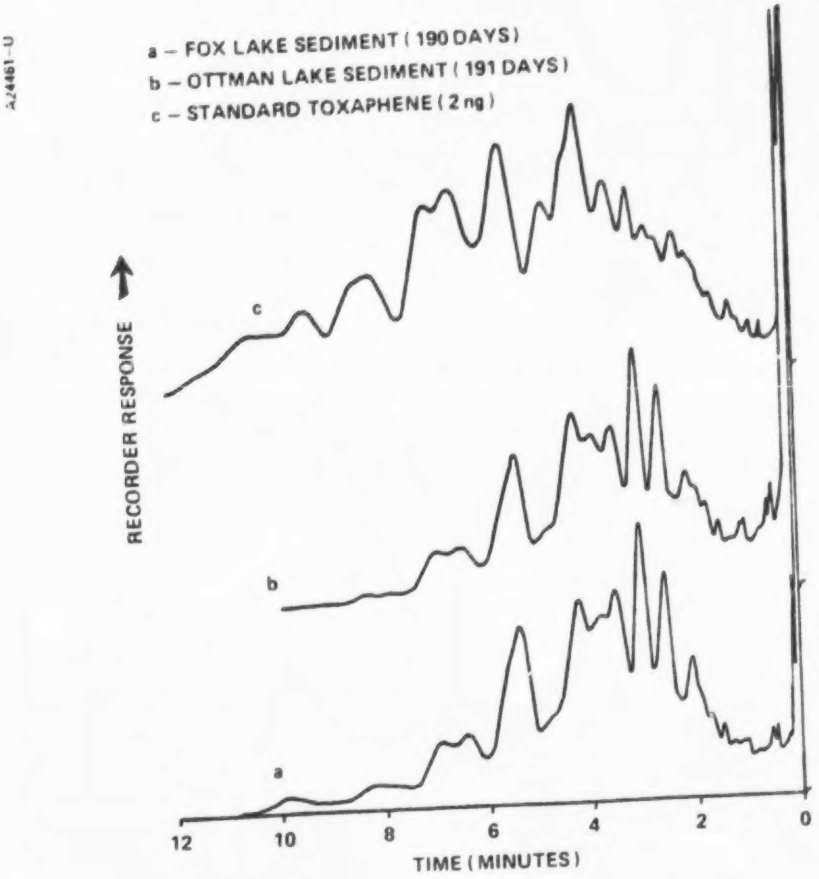

Figure 7.5. GC patterns of toxaphene from sediments of Fox Lake and Ottman Lake compared to standard toxaphene. Source: Hughes, 1970.

peaks near the end of the pattern with increasing strength of the early eluting peaks (Hughes, 1970). This same effect occurs when standard toxaphene is dehydrohalogenated. Hughes (1970) ruled out a dehydrohalogenation process for the formation of residue toxaphene by demonstrating that residue toxaphene is unaffected by nitration, whereas dehydrohalogenated standard toxaphene is altered by nitration.

In summary, the available information suggests the label "hard," as used with dieldrin and DDT, does not apply to the persistence of toxaphene residue toxicity in water (Hughes, 1970). Also, the persistence of toxaphene in the chemical sense also appears to be somewhat less than that of other organochlorine pesticides. For example, the concentration in sediment decreases by a factor of 2 every 20 days. Nevertheless, detectable quantities of toxaphene are found in sediment and water 3 to 9 years after treatment of a lake. Concentrations of toxaphene in water rapidly decrease because the chemical is absorbed on suspended particulates and sediment.

7.3.3 Mobility and Persistence in Soil

Toxaphene has infrequently been detected in soils which have not been exposed to the pesticide during crop protection (Section 7.2.3). For example, Wiersma et al. (1972 b) detected toxaphene in only one soil sample out of 199 non-crop samples. A study of soils in 8 cities detected toxaphene in 4 out of 400 sites and only in southern cities. This suggests that the major source of soil toxaphene residues is from its direct use as a pesticide, mostly for the protection of cotton.

Estimates of the half residence time for toxaphene in soil vary from 100 days (LaFleur et al., 1973) to 11 years (Nash and Woolson, 1967). Because of the experimental conditions used, Nash and Woolson (1967) expected that their results provided an upper limit of persistence. In 1951 they applied toxaphene to Congarec sandy loam soil in approximate concentrations of 55.8 and 137.0 ppm (Nash et al., 1973). The plots were surrounded by concrete walls and the pesticide was thoroughly mixed before being placed to a depth of 38 cm and provided with gravel and tile drainage. After 14 years, 45 percent of the toxaphene remained in the soil. The authors felt that the treatment and maintenance of the soil samples would allow minimum leaching, volatilization, photodecomposition and mechanical losses because of the thorough mixing and minimum tillage and probably little biological decomposition because of the high rate of application which may have been toxic to the soil microorganisms. Twenty years after treatment Nash et al. (1973) used the same plot to compare the efficiencies of three methods (shake, Soxhlet, and column) for extracting toxaphene from soil. By taking the mean value for the Soxhlet and shake extraction recoveries at both treatment rates, they determined that 45.1 percent of the total amount of toxaphene residue remained after 20 years.

LaFleur et al. (1973) used a 10 x 10 meter plot of Dunbar soil which overlay a water table that was usually less than 3 meters from the surface. Toxaphene was sprayed at a rate of 100 kg/ha and then hand raked into the soil. They plotted the change in concentration with time and determined that the loss was rapid at first but slower after 80 days. The calculated half residence time was 100 days (32 ppm to 4 ppm in 1 year).

The residence time of toxaphene in soil has been reported by a number of other investigators. Westlake and SanAntonio (1960) found that the levels of toxaphene in air-dried soil decreased from 140 to 85 ppm over a six year period. Adams (1967), using the data of Foster et al. (1956), has calculated the approximate half-life in years of various pesticides in three soils. The values for toxaphene were 2.0, 0.8, and 0.8 for Beltsville, Mississippi, and New Jersey soils, respectively. Hermanson et al. (1971) followed for 9 years the concentration of toxaphene in a Holtville sandy clay that received a treatment of 22.4 kg/ha for 4 years followed by no treatment for 5 years. They rated toxaphene as 0.18 on a persistency index where 1.00 was equal to no degradation during the first year (DDT = 0.26). They also calculated a persistence half-life of 4 years which was the same as the value for DDT. A slight correlation between persistence and vapor pressure was noted. Seiber et al. (1975) analyzed soil samples from a California cotton field 6 and 12 months after spraying. They found a concentration of approximately 5 ppm at 6 months and approximately 3.5 ppm at 12 months. Bradley et al. (1972) found that the toxaphene residue in acidic loam declined during the year sampling period while the DDT residue stayed basically the same.

This range of residence times may be explained by climate and soil factors. Chemicals can be degraded or lost from soil by volatilization and codistillation, leaching, oxidation, hydrolysis, and microbial degradation (Edwards, 1966). Some photodegradation is possible but only on the top surface. These processes are affected by climate and soil factors, such as soil type, pH, organic matter content, mineral (especially clay) content, drainage and soil moisture, and cropping (Edwards, 1966; Adams, 1967). Although the effect of these factors cannot be quantitatively correlated, some general qualitative statements are possible. Heavy clay soils appear to increase the persistence of chlorinated hydrocarbons (Harris, 1966; Edwards, 1966) and temperature increases result in increased rates of loss (Edwards, 1966). Little evaporation occurs from dry soil (which may explain the results of Westlake and SanAntonio, 1960) while considerable evaporation occurs from wet soil (Adams, 1967). Biodegradation may increase on addition of carbon sources such as manure because of the increase in metabolic activity of the microorganisms (Adams, 1967). Edwards (1966) has listed the following factors affecting soil insecticide persistence in decreasing order of importance:

(a) the chemical structure of the insecticide;

(b) the type of soil to which the insecticide is applied, especially depending on the organic matter content; amount of rainfall reaching treated soil and the moisture in the soil;

(c) the microbial population of the treated soil; depth of cultivation of the insecticide; mean temperatures in the treated soil;

(d) the mineral content and acidity of the treated soil; amount of plant cover over treated soil; formulation and concentration of the insecticide.

The effect of these environmental factors on the fate of toxaphene in soil has been studied by only a few investigators. In a recent study, Parr and Smith (1976) studied the degradation of toxaphene in Crowley silt loam (pH 6.0) under a variety of conditions. They used both aerobic and anaerobic conditions as well as a sterilized sample that was later inoculated with microorganisms. Both the loss of toxaphene and the cumulative release of carbon dioxide were measured. In order to see the effect of organic matter, some samples were amended with alfalfa meal. The percent toxaphene remaining (starting concentration 10 ppm) after various times is depicted in Figure 7.6. The results show that no degradation occurs under aerobic conditions, while substantial breakdown occurs under anaerobic conditions. Addition of extra

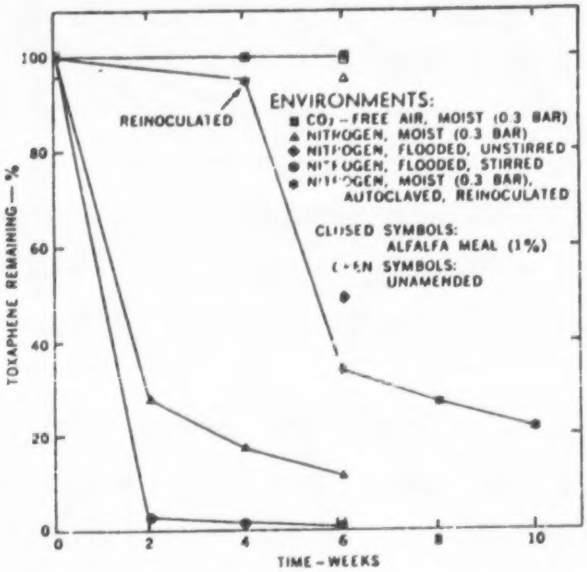

Figure 7.6. Degradation of toxaphene in soil unamended or amended with alfalfa meal and incubated in a moist aerobic environment or under moist and flooded anaerobic conditions. Source: Parr and Smith, 1976.

organic matter has a positive effect on degradation under anaerobic conditions. It appears that microorganisms are degrading the toxaphene since no loss occurs in a sterilized, anaerobic sample until it is inoculated. Plimmer (1974) reported that anaerobic conditions, such as flooded soils or sediment, appear to accelerate reductive metabolism. Parr and Smith (1976) also measured the redox potential and concluded that degradation is very rapid between 0 to -100 mV.

Leaching of toxaphene through soil has been examined by several researchers. Swoboda and coworkers (1971) analyzed three plots of Houston black clay soil (50 to 60 percent montmorillonitic clay) which received a total of 11.2, 20.16, and 24.64 kg/ha. The percentage of the applied toxaphene recovered in the soil varied from 10 to 20 percent, depending upon the rate of application. By measuring the concentration of toxaphene at various depths, it was determined that 88 to 95 percent of the toxaphene was found in the top 12 inches (measured by foot increments down to 60 inches). The authors concluded that very little leaching occurred. Nash and Woolson (1968) reported similar results with toxaphene residues measured in Congarec sandy loam soil 15 years after application. They found 85 percent of the toxaphene in the upper 23 cm, which probably corresponds to the cultivated layer. A study of toxaphene desorption from soil using various solvents indicated that little toxaphene would be desorbed from soil by water (LaFleur, 1974).

Although all the above information suggests that toxaphene does not leach through soil, LaFleur and coworkers (1973) found that toxaphene was leached through Dunbar soil overlying a water table less than 3 meters from the surface. This may have been assisted by the high total rainfall encountered (13 percent higher than mean for the past 72 years).

The ability of toxaphene to migrate from the surface to ground water was suggested by the work of Johnston et al. (1967). They detected toxaphene in flooded plots where they were studying the movement of other pesticides. Since they had not used toxaphene, they concluded that the toxaphene was from contaminated ground water.

Bradley et al. (1972) examined the pesticide runoff from two locations each of which were either treated with toxaphene (26.8 kg/ha) or a combination of toxaphene (268 kg/ha) and DDT (13.4 kg/ha). Only 0.3 to 0.6 percent of the applied toxaphene was detected in the runoff. Of that recovered, 75 percent was associated with sediment (96 percent with DDT). The amount in the runoff was dependent upon climatic factors such as frequency, intensity, and duration of rain and soil physical properties. The toxaphene runoff into a farm pond was enough to exceed the 96 hour medium tolerance limit for blue gill (3.5 ppb).

Only two studies have discussed the alteration of the gas chromatographic fingerprint resulting from the weathering of toxaphene in soil (Parr and Smith, 1976; Seiber et al., 1975). Seiber et al. (1975) found that the less volatile components (longer retention time components) tended to be enriched. This was similar to what was found with residues on foliage which was attributed to selective evaporation. The effect in soil may be attributed to drainage from leaves since the residue concentration in soil increased at first.

In contrast, Parr and Smith (1976) found a diminution of the later peaks when toxaphene was incubated with soil under anaerobic conditions. This result is similar to that which Hughes (1970) found with toxaphene residues in water and sediment.

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8.0 ENVIRONMENTAL INTERACTIONS AND THEIR CONSEQUENCES

8.1 SUMMARY

Toxaphene is released into the environment primarily from its application as an insecticide for the protection of cotton, mostly in the southern states. Present production of toxaphene is estimated at 49 million kg per year. Toxaphene sprayed on cotton either evaporates or is deposited upon soil or foliage. Toxaphene in air can travel for long distances (1200 km). Residues in soil will evaporate, degrade, or, depending upon the soil and climate conditions, sometimes will leach into the ground water. Evaporation from water or adsorption to sediment or suspended solids appear to be the primary mechanisms for removal of toxaphene from water. Human exposure to toxaphene through food or terrestrial sources appears to be very limited, although some residues have been detected in birds, wildlife, and food products. Bioconcentration of toxaphene in aquatic organisms is very sizable (in fish up to 75,000 times the concentration found in water) and may result in human exposure and residues in fish-eating birds.

8.2 SOURCES OF TOXAPHENE

Toxaphene is a complex mixture of polychlorinated diterpenes which is produced by the chlorination of the diterpene, camphene. It is now the most commonly used chlorinated hydrocarbon insecticide. While release to the environment primarily occurs when it is applied for pest control, some is released during manufacture from formulating plants and from accidental spills (Gerakis and Sficas, 1974). There are no known natural sources or inadvertant, man-made sources of toxaphene.

8.2.1 Toxaphene Production and Consumption

Since the agricultural chemical industry will not release precise figures on production or consumption for proprietary reasons (VonRumker et al., 1974), the available data are only estimates. Table 8.1 describes production by manufacturer and plant location for the years 1972 and 1975. The largest production plant is the Hercules Inc. plant located at Brunswick, Georgia. Other plants which have large manufacturing capacity are the Tenneco Chemicals (Fords, NJ) and Sonford Chemical Co. (Houston, TX). As a result of the environmental regulations limiting the use of persistent, organochloride insecticides, toxaphene production and consumption has been rapidly increasing. Ifeadi (1975) projects that production will reach 105.9 million kilograms of active ingredient in 1980 (compared to 49.1 million kilograms in 1975) based on the present market conditions.

Toxaphene mainly enters the environment through application as an insecticide. It is consumed in distinct regional and seasonal patterns. It is primarily applied in the southern states and in California for the control of pests infesting cotton farms (Guyer et al., 1971; Hartwell et al., 1974; VonRumker et al., 1974). Although the amount used for other farming applications is small, significant amounts of toxaphene are still used as a livestock insecticide. In the past, toxaphene has been used for rough fish control in lakes. Table 8.2 and Figure 8.1 describe the consumption by geographical region for 1972 (VonRumker et al., 1974). Hartwell et al. (1974) have suggested that the consumption in California might be understated. While VonRumker and co-workers have suggested that about 8 percent of the toxaphene is consumed in California, Bailey and Hannum (1967) have stated that about 20 percent of the total pesticides for the U.S. are consumed in California.

Seasonal variation in toxaphene consumption follows the need for its application in its major use, cotton farming. Several investigators (Nicholson et al., 1966; Tabor, 1966; Stanley et al., 1971; Willis et al., 1976) have concluded that the usual time of application for toxaphene ranges from late

Table 8.1 Toxaphene production[a]

Manufacturer	Location	Capacity (millions of kilograms)	Production (millions of kilograms) 1972	Production (millions of kilograms) 1975
Hercules, Inc. Synthetics Dept.	Brunswick, GA	22.7-34.1	25.4	29.5
Tenneco Chemicals Intermediates Div.	Fords, NJ	56.8	9.1	9.1
Sonford Chemical Co.	Houston, TX	18.2	0	9.1
Vicksburg Chemical Co.	Vicksburg, MS	2.3	0	1.4
TOTAL			34.5	49.1

[a] Source: Adapted from VonRumker et al. , 1974 and Ifeadi, 1975.

Table 8.2 Toxaphene consumption in 1972[a]

Distribution of Toxaphene	Consumption (millions of kilograms)
U.S. Production	34.5
Imports	0
Exports	8.2
U.S. Consumption	26.3

Region	Agricultural	Industrial Commerical	Total
Northeast	Negl.	0.1	0.1
Southeast	9.1	0.1	9.2
North Central	0.5	0.1	0.5
South Central	14.3	0.1	14.4
West	2.0	0.1	2.1
TOTAL	25.9	0.5	26.3

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[a] Source: VonRumker et al. ,1974.

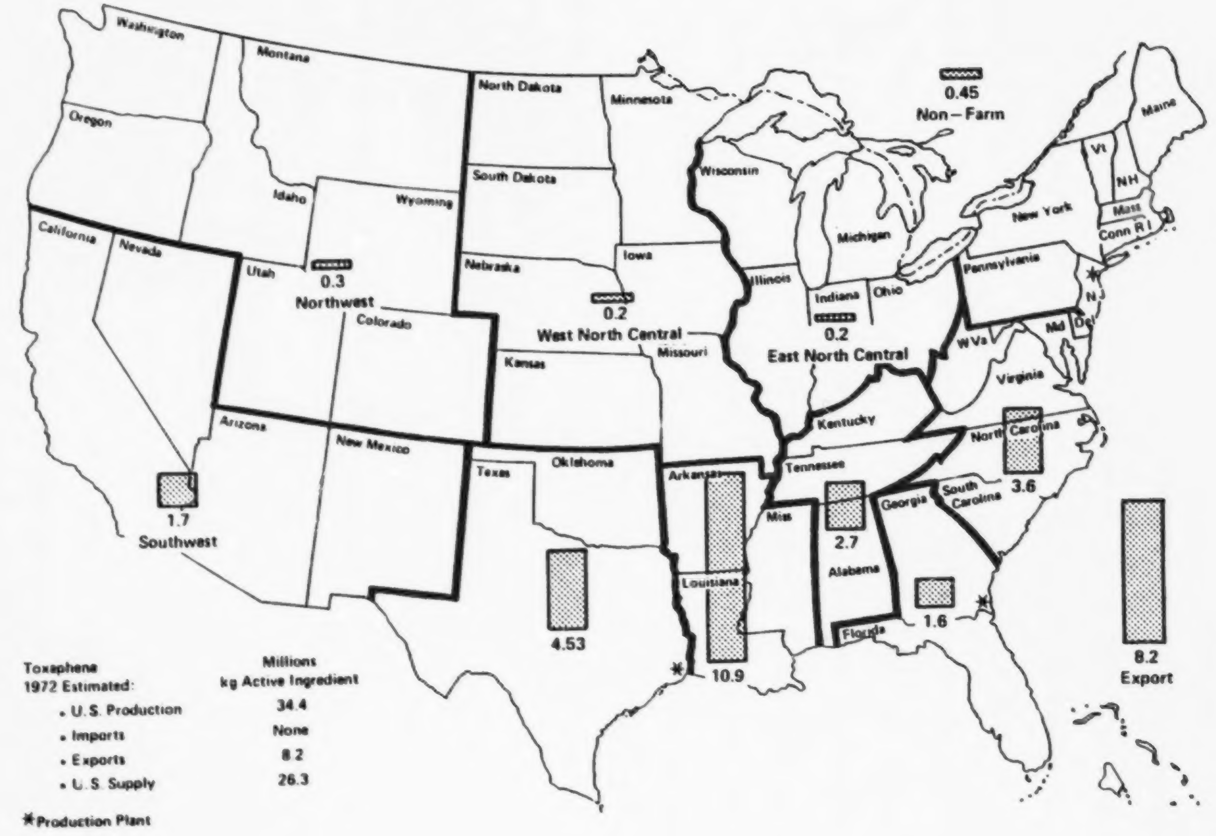
C24582-U
Washington
Oregon
California
Nevada
Idaho
Montana
Wyoming
Utah
Colorado
Arizona
New Mexico
Texas
Oklahoma
Kansas
Nebraska
South Dakota
North Dakota
Minnesota
Iowa
Missouri
Arkansas
Louisiana
Wisconsin
Illinois
Michigan
Indiana
Ohio
Kentucky
Tennessee
Miss
Alabama
Georgia
Florida
South Carolina
North Carolina
Virginia
W Va
Md
Del
Pennsylvania
New York
N J
Conn
R I
Mass
NH
Vt
Maine
1.7
Southwest
0.3
Northwest
0.2
West North Central
0.2
East North Central
0.45
Non – Farm
4.53
10.9
2.7
1.6
3.6
8.2
Export
Toxaphene
1972 Estimated:
Millions
kg Active Ingredient
• U.S. Production 34.4
• Imports None
• Exports 8.2
• U.S. Supply 26.3
*Production Plant

Figure 8.1 Materials flow diagram for toxaphene, 1972. Source: von Rumker et al., 1974.

July into early August. This period corresponds to the incursion by boll weevils and bollworms. Toxaphene is sometimes applied in the spring to control cutworm infestation.

8.3 ENVIRONMENTAL CYCLING OF TOXAPHENE

A variety of degradative and transport processes may effect the environmental fate of toxaphene (Section 7.3). Intermedia processes which affect the environmental cycling of toxaphene include evaporation, water leaching from soil, adsorption to soil followed by erosion, washout by rain, and adsorption to particulate matter followed by precipitation (effective in both water and air). The high vapor pressure of commercial toxaphene, compared to other organochlorine pesticides, assures that atmospheric processes are extremely important. Because commercial toxaphene consists of numerous components, these intermedia processes have considerable potential for altering the chemical composition of the toxaphene residue.

Quantitative information on the cycling of toxaphene is not available. Most studies have been closely tied to a particular release source; (1) conventional crop (cotton) spraying, (2) treatment of lakes for rough fish control, or (3) loss in water from production plants.

Toxaphene sprayed on crops will be either deposited on the foliage or soil or will evaporate. Air monitoring information (Arthur et al., 1976; Stanley et al., 1971; Tabor, 1965, 1966) indicates that atmospheric residues of toxaphene are highest in the southern cotton belt and that the residue concentrations are highest during the months of July and August when the greatest rates of application occur. The evaporated toxaphene, probably as a vapor, although it might adsorb to particulate matter, can travel long distances (1200 km) and become well mixed in the atmosphere (Bidleman and Olney, 1975). Monitoring of toxaphene residues in air at locations remote from application sites indicate that the chemical makeup of toxaphene is only slightly altered. In contrast, Seiber et al. (1975) found that toxaphene detected above a cotton field showed a substantial enrichment of the more volatile components. The remote monitoring results suggest that significant amounts of all the toxaphene components may be lost to the atmosphere during spraying. Swoboda et al. (1971) in their field study, could only recover 10 to 20 percent of the applied toxaphene in the soil.

Of the sprayed toxaphene that reaches the foliage and soil, substantial amounts appear to evaporate. Seiber et al. (1975) concluded that the more volatile components which are deposited on foliage evaporate, while the less volatile components are washed off and drop into the soil. In contrast, in soil, the percentage of the more volatile components increases, suggesting some type of degradation of the less volatile components. Carlin et al. (1974) found no evidence of metabolism of 36Cl-toxaphene applied to cotton plants maintained in a closed all-glass system for 7 days. Thus, the mechanism of loss was volatilization.

Toxaphene residues in soil may be transported by erosion, leaching, or evaporation. The rate of toxaphene evaporation from soil has not been studied, but is likely to be dependent upon a variety of soil factors, especially soil moisture (Edwards, 1966). Bradley et al. (1972) found that 0.3 to 6.0 percent of the applied toxaphene was detected in the runoff (75 percent associated with sediment). Monitoring of a watershed which drains an agricultural area containing small cotton farms (Nicholson et al., 1964) also indicates that water contamination may result from runoff. Whether toxaphene residues in soil can leach into ground water is probably dependent upon the particular soil and climate conditions. Swoboda and coworkers (1971) determined that 88 to 95 percent of the toxaphene residue was found in the top 30.5 cm (measured by foot increments down to 152.4 cm). Similar results were found by Nash and Woolson (1968). However, LeFleur et al. (1973) found that toxaphene was leached through soil overlying a water table less than 3 meters from the surface. Johnston et al. (1967) also found suggestive evidence that toxaphene from contaminated ground water could migrate into flooded plots.

Considerable study of lakes treated with toxaphene for rough fish control suggest that toxaphene is removed from water by evaporation or codistillation and by adsorption to sediment or to suspended particulate matter, followed by settling. Shallow, biologically rich lakes become detoxified (in respect to fish) faster than deep, biologically sparse lakes (Terriere et al., 1966), which is consistent with a toxaphene adsorption mechanism (Veith and Lee, 1971). However, monitoring data from river systems suggests that adsorption may not reduce the concentration of toxaphene in river water. For example, Grzenda and Nicholson (1965) found concentrations of toxaphene in river water varying from 30 to 140 ppt, but no toxaphene in river bottom sediment. In contrast, Bailey and Hannum (1967) found higher concentrations of toxaphene in sediment than in water. The calculated half-life for toxaphene evaporation from water is 10.4 hours, and monitoring studies by Veith and Lee (1971) have suggested that greater than 50 percent of the toxaphene applied to lakes is lost through evaporation or microbial degradation. As with soil residues, toxaphene residues detected in water systems have a decreased percentage of less volatile material, but it has been shown that this is not due to dehydrochlorination (Hughes, 1970).

Reimold and Durant (1972) have studied toxaphene residues in water systems which receive effluents from the Hercules production plant in Terry Creek, Brunswick, Georgia. They concluded that toxaphene was confined to suspended material in the water and the concentration of residues in the sediment decreased with an increase in distance away from Terry Creek. These results again suggest that adsorption to sediment may be an important process for removing toxaphene from water.

8.4 FOOD CHAINS

8.4.1 Toxaphene in Food

Human ingestion of toxaphene in food is apparently low. The annual market basket survey conducted by the Food and Drug Administration during a four year period (Table 4.6) showed that most products contained no detectable concentrations of toxaphene. The only exceptions were leafy vegetables and garden fruits, both of which had very low levels (calculated intake of approximately 0.001 mg per day). The low concentrations in food are probably due to the fact that toxaphene is mostly used on cotton crops, and washing and food processing remove considerable amounts of toxaphene (Van Middlelem, 1966; Van Middelem and Wilson, 1960; Hartwell et al., 1974). Toxaphene residues have been detected in tobacco products (Domanski et al., 1975), probably as a result of drift from cotton spraying in nearby fields.

8.4.2 Terrestrial Ecosystems

Bioaccumulation of toxaphene residues in terrestrial ecosystems is not well understood. Uptake and adsorption of toxaphene in plants has not been studied, although residues have been detected in some instances. These residues are apparently due mostly to surface deposition (drift or intentional spraying), rather than uptake from soil (Hartwell et al., 1974). Little information on adsorption and distribution in birds and terrestrial wildlife is available, but residues have been detected in some species. For example, in a study in Alabama, Causey et al. (1972) detected toxaphene in 5 of 20 (average concentration 30 ppm) quail, and detectable concentrations were found in 2 of 31 rabbits and 3 of 22 deer. Toxaphene residues have been detected in fish-eating birds (Keith, 1965) and in non-fish-eating birds (Markley, 1974), and the passage of toxaphene into the eggs of wild birds has been documented.

Toxaphene residues have not been detected in domestic animals except in controlled exposure studies. Several studies have demonstrated that cows fed toxaphene in their diet (Zweig et al., 1963; Claborn et al., 1963) or in treated hay (Bateman et al., 1953) have residues in their milk. However, toxaphene has not been detected in milk in the annual market basket survey (Section 8.4.1).

8.4.3 Aquatic Ecosystems

Toxaphene appears to have considerable potential for bioaccumulating in aquatic organisms, resulting in possible exposure to humans. Aquatic plants are able to bioaccumulate toxaphene from the water (Section 4.0). For example, Terriere et al. (1966) found 15.5 ppm toxaphene in aquatic plants which were exposed to water containing 2 ppb. Other levels of the food chain, such as bacteria, fungi, and green algae (Paris et al., 1975), are also capable of bioaccumulating toxaphene. Bioconcentration of toxaphene in aquatic invertebrates, such as oysters (Lowe et al., 1971), plankton (Johnson, 1966), and daphnia (Schoettger and Olive, 1961), has also been documented.

Several studies have indicated that toxaphene is bioconcentrated in fish by a factor of several thousand. This conclusion has been supported by several monitoring studies (Kallman et al., 1962; Terriere et al., 1966;

Hughes and Lee, 1973) and laboratory investigations (Hughes, 1970; Sanborn et al., 1976; Mayer et al., 1975). Sanborn et al. (1976) exposed mosquito fish for three days to 44.4 ppb toxaphene (C-14 labelled) in a model ecosystem and found a concentration factor of 4247. Mayer et al. (1975) have demonstrated that toxaphene can be concentrated up to 75,000 times in fish, with the rate appearing to be dependent upon the lipid concentration in the fish. Hughes and Lee (1973) found that the concentration in prey fish stocked in a toxaphene treated lake was more than the concentration in predator fish, which suggests that biomagnification may not be an important process. The bioconcentration of toxaphene in fish is further supported by numerous field monitoring studies (Table 5.6) which have detected toxaphene in fish samples. However, the levels that have commonly been detected are relatively low and in some instances no toxaphene is detected. For example, Reimold and Shealy (1976) monitored chlorinated pesticide residues in fish collected from 11 estuaries representing all the Atlantic drainage basins in Georgia and South Carolina. Although they detected dieldrin, DDT, and PCB's, no measurable residues of toxaphene were found even though a toxaphene manufacturing plant was located in New Brunswick, Georgia.

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